DOES AN INTEGRATED MEASURE OF CORTICOSTERONE FROM FEATHERS IMPROVE OUR UNDERSTANDING OF AVIAN ECOPHYSIOLOGY?

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

in the Department of Biology

University of Saskatchewan

Saskatoon

by

Graham David Fairhurst

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

DISCLAIMER

Reference in this thesis/dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Biology
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5E2
Canada

OR

Dean
College of Graduate Studies and Research
University of Saskatchewan
107 Administration Place
Saskatoon, Saskatchewan S7N 5A2
Canada

ABSTRACT

The hormone corticosterone (CORT) helps mediate the relationship between birds and their environment. I studied nestling and adult birds in several diverse contexts to understand how sources of environmental variability contribute to variation in an integrated measure of CORT from feathers.

In nestling tree swallows (*Tachycineta bicolor*), feather CORT was positively related to maximum nest box temperatures. Temperatures may not have been challenging for nestlings. Instead, CORT physiology was likely matched to nest box conditions. Experimentally-reduced provisioning resulted in significantly lower feather CORT in nestling Cory's shearwaters (*Calonectris diomedea*), suggesting nestlings invoked an adaptive strategy of CORT suppression to cope with extended nutritional challenges.

In adults, both enrichment and its removal from the cages of captive Clark's nutcrackers (*Nucifraga columbiana*) resulted in significant increases in feather CORT, but this effect was a function of exposure time. In adult tree swallows, increased productivity was positively associated with CORT from feathers grown post-breeding. Although this effect likely reflected the increased energetic expense of raising more young, it could not be separated from individual quality. Traditional measures of habitat and spatial structure related to Dupont's lark (*Chersophilus duponti*) population dynamics were not related to feather CORT. However, when ratios of stable isotopes of carbon ($\delta^{13}C$) were used as a proxy for environmental conditions, a significant negative relationship resulted between $\delta^{13}C$ and feather

CORT. This result suggests that combining feather-based measurements is a particularly strong approach to studying habitat-physiology relationships.

Feather CORT quantified hormonal responses to ecological variability in general, rather than in response to any specific type(s) of challenges. A unifying theme of my work was that, when interpreted in proper context, feather CORT was apparently related to energetic demand or exertion. Feather CORT may therefore be a proxy for individual energetics. Building on this, I developed a conceptual model that helps explains how environmental and physiological variation delineates an "ecophysiological niche", the boundaries of which define a range of CORT values that should contribute positively to fitness. My results suggest that feather CORT will likely be useful in moving both theoretical and applied research towards a more holistic perspective of avian ecophysiology.

ACKNOWLEDGEMENTS

To say that this thesis has been a collaborative effort does not capture the spirit of the work. I benefitted immeasurably along the way from numerous colleagues and collaborators, family members, and friends. First, I owe a debt of gratitude to my late supervisor, Dr. Gary Bortolotti, for his guidance, wisdom, and friendship. I only wish that I could thank him in person. I also want to thank my co-supervisor, Dr. Tracy Marchant, for her guidance throughout the years and particularly for her physiological knowledge; both were instrumental to my success. Second, I am very grateful to my committee members (Drs. Keith Hobson, Debbie Kelly, and Karen Wiebe) for their input. I have been extremely lucky to have such a great group of scientists guiding my professional development. Third, I thank the University of Saskatchewan and the Department of Biology for providing both the financial and academic support that made my work possible. I was generously supported by a Malcolm A. Ramsay Memorial Award, a Candace Savage and Keith Bell Fellowship in Grasslands Ecology Studies, a Dr. Ruby Larson Student Research Award, and a Nature Saskatchewan Graduate Student Scholarship. Other members of the lab always provided stimulating discussion and good friendship. I would especially like to thank Drs. Matthias Vögeli, Manu Grande, and Sonia Cabezas for helping improve my thinking, writing, and statistical power, and Marten Stoffel for hours of fun in the field and office. I benefitted from conversations with numerous faculty, staff, and graduate students over the years, and I want to thank Vanessa Harriman and Dr. Bob Clark in particular. Fourth, I have a few random thank-yous: to Dr. Marc Bechard for providing me with the stepping-stone that led me to

Saskatoon and to Dr. Al Dufty, Jr., for exposing me to behavioural endocrinology – both have been life changers – and also to Armin van Buuren for providing the soundtrack to my writing. Finally, I wish to thank my partner, Leanne Schinkel, for everything; I could not have done this without her.

DEDICATIONS

I dedicate this thesis to my parents, for always giving me the freedom to forge my own path, teaching me to recognize the difference between failure and not succeeding, and providing endless love and support. Thank you!

I also dedicate this thesis to my late supervisor, Dr. Gary R. Bortolotti, for his commitment to me and our collaborative work. His friendship and sage advice are greatly missed. This work would not have been possible without him, and I am proud to be a part of his legacy.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. General introduction

1.1. Introduction

Vertebrates live in a dynamic world and must respond appropriately to their environment to maximize fitness [1]. Understanding the ecological factors to which vertebrates respond and how responses relate to fitness are of central importance to ecology [2]. Insight into these relationships can be gained by studying the physiological and behavioural mechanisms that comprise such responses [3,4]. Products of the endocrine system (i.e., hormones) coordinate transitions through life history stages and responses to both internal and external change. Therefore, hormones play an important role in orchestrating the physiological and behavioural changes required to survive, grow, and reproduce [5-7].

The hypothalamic-pituitary-adrenal (HPA) axis is particularly influential because it acts quickly in response to environmental challenges to help maintain homeostasis [2,8-11]. When the HPA axis is activated, a hormone cascade begins in the hypothalamus with the release of corticotropin-releasing hormone (CRH). Within seconds, CRH travels to the anterior lobe of the pituitary, where it stimulates release of adrenocorticotropic hormone (ACTH) into systemic circulation. ACTH acts on the adrenal cortex to release glucocorticoid hormones (GCs) within minutes [6].

1.2. Glucocorticoid hormones in ecology

Products of the HPA axis, especially GCs, have long been known to play an important role in the health of vertebrates (e.g., [12]). Considerable research over the past few decades has elucidated the mechanisms of GC action and the numerous influences GCs have on the physiology of vertebrates [13]. More recently, interest in GCs from behavioural and ecological perspectives has led to productive cross-discipline research, particularly with birds. GCs lend themselves well to these perspectives for three reasons.

First, GCs regulate energy balance through their effects of reorganizing and mobilizing energy substrates such as fat and protein [14-16]. Importantly, energy is a currency of ecology [17], and how individuals allocate limited energetic resources between competing demands contributes to life-history trade-offs [18,19]. GCs promote conversion of fat depots into triglycerides and protein stores into amino acids [20,21]. Additionally, GCs increase production of glucose from amino acids (i.e., gluconeogeneis) in the liver and oxidation of fatty acids within cells [20]. GC levels are therefore associated with increased glucose and plasma triglyceride levels [15,21]. Overall, GCs shift energy use from carbohydrate sources to use of fat stores and protein. GCs cycle on a daily basis [22] and vary seasonally [23-25] and with factors related to latitude [26-28], suggesting that they are integral to avian life-history [11,18,29,30].

Second, GCs are released to help the animal cope with unpredictable or noxious stimuli (i.e., stressors [2,13,31]). Ecological factors such as predation, severe weather, diet quality and quantity, social conflict, parasite infestation, and habitat quality—all of which can act as selective pressures—can influence activity of the HPA axis [32-41]. Differences in

how individuals respond to similar stressors can be consistent among individuals [42-44] and there appears to be a genetic basis for this [45], suggesting that selection may be acting on GC responses [27]. Challenges to homeostasis are pervasive in the wild and evidence clearly suggests that GCs are important in coping with such challenges.

Third, GCs are related to physiology and behaviour throughout the life cycle. For example, in young birds, corticosterone (CORT, the primary avian GC) is related to begging behaviour [46,47] and dispersal [48,49]. CORT can promote fall migratory behaviours such as increased activity and migratory fattening [50-52]. There are cognitive benefits to increased GCs [53], such as enhanced memory during winter in seasonal food caching species [54,55]. Yet there are also detrimental cognitive effects, including neuronal death, in other contexts [13,56]. Consistent patterns in how CORT relates to fear responses, exploration, and novelty suggest a role for GCs in avian personality [44,57,58].

A complex relationship also exists between GCs and the immune system [13,59,60]. For example, increased CORT in eggs can result in suppression of some aspects of the immune response in nestlings [61], and nestling immune response can negatively covary with CORT [62], yet small amounts of CORT during development may increase immune function later in life [63]. The interplay of GCs, immune response, and other physiological systems has led to the incorporation of responses to stressors into models of honest signalling [64-66].

GCs are also related to reproduction. The effects of GCs and other products of the HPA axis include suppression of reproductive function [13,67] and an interaction with prolactin to alter parental care [68,69]. Yet, other studies show that parental effort is positively correlated with GCs measured during [70,71] and after [72] reproduction. Pre-

and post-natal GCs can have permanent organizational effects [73]. For example, conditions experienced by female birds can influence levels of CORT deposited in eggs [74,75]. CORT *in ovo*, in turn, can alter development of the offspring's GC responses [76], sex ratio [71,77,78], flight performance [79], and immune response [77]. Similarly, elevated CORT in post-natal offspring can influence dominance and incubation behavior [80,81], and GC responses [82,83], later in life. Although not always causal, GCs are meaningfully connected to many aspects of avian behaviour and physiology, including components of fitness ([84-86]; for reviews see [70,87]).

1.3. Addressing knowledge gaps

Although GCs clearly have many important functions in vertebrates, the relationships between GCs and life-history stages, survival, and reproduction is still poorly understood [11,25,70,87]. These unresolved issues in GC ecophysiology are due to multiple contributing factors. One reason is that GC studies often do not directly assess performance and fitness measures [87], and logistical constraints can make collecting these data difficult. Additionally, categorical consideration of GC data (i.e., baseline vs. stress-induced), as is typical in most studies using plasma GCs, and interpretation of high levels of GCs as "stress", may be simplistic. Finally, methodology limits the types of GC data that can be collected and, additionally, the time frame over which they can be collected.

In my thesis, I attempt to present a new and broader perspective on GCs based on a new approach to collecting GCs and a recent theoretical model (i.e., Bortolotti et al. 2008, Romero et al. 2009). I hope that a broader perspective will lead to a more unified theory of

GC ecophysiology and a more holistic appreciation of GCs that goes beyond simple "baseline" and "stress-induced" categories that can obscure significant individual variation [88]. Although it is important to recognize that GCs at increasing levels have different physiological effects, the time course over which they act must also be considered [15,89]. However, with few exceptions (e.g., social stressors; [37,90]), we have little appreciation for how frequently vertebrates encounter stressors in the wild, how long stressors last or, by extension, how frequently maximum levels of GCs occur compared to levels at a baseline.

1.4. Instantaneous vs. integrative sampling of corticosteroids

Instantaneous blood samples, even when taken over hours, still provide only a short time frame of GC physiology and may not be repeatable, even over short periods [91]. Instantaneous sampling may be meaningful for short-lived stressors, but less so for longer-term stressors, life-history periods, and ecological factors [91]. Indeed, it is over longer time periods that the deleterious effects of chronically elevated GCs arise [13]. Despite these uncertainties, strong biological significance is attributed to stress-induced GC levels when baseline may actually be more important, especially in mediating life-history transitions [11]. GCs at all levels and temporal scales are essential to survival. However, the need to account for considerable individual variability in the magnitude and repeatability of both baseline and stress-induced values [88,91], and the time course over which GCs act, suggests a more holistic approach to the ecophysiology of GCs may be warranted.

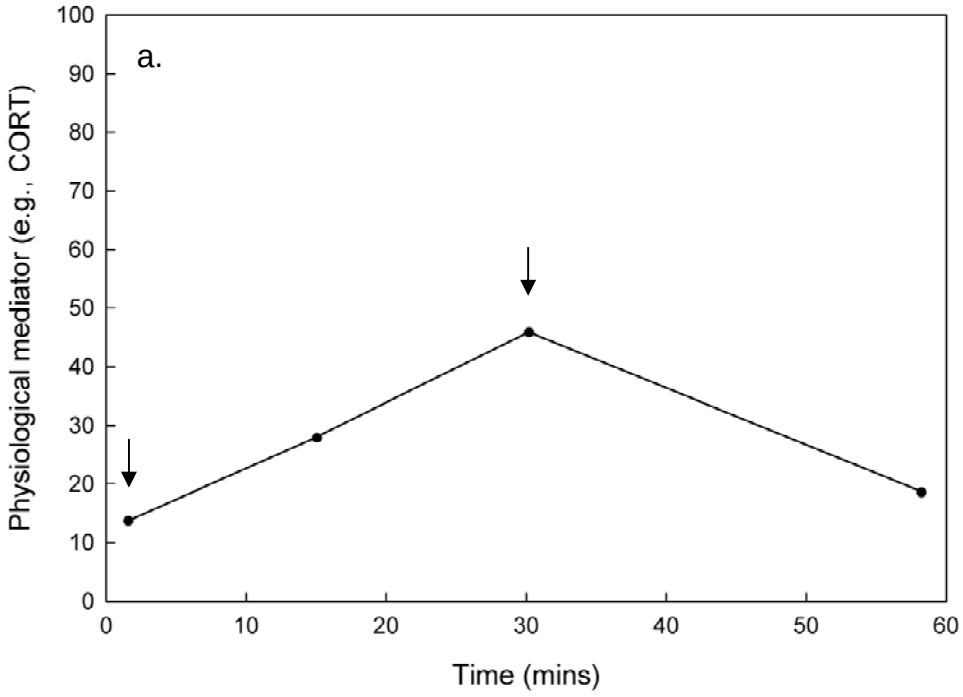
Integrated measures of GCs may provide new insights because they do not distinguish between baseline and stress-induced GC values. Instead, they incorporate both the amplitude and duration of hormone secretion into one value. Integrated responses can be calculated from blood samples (e.g., [22,42,92]) and have the benefit of lower inter-individual variability [92].

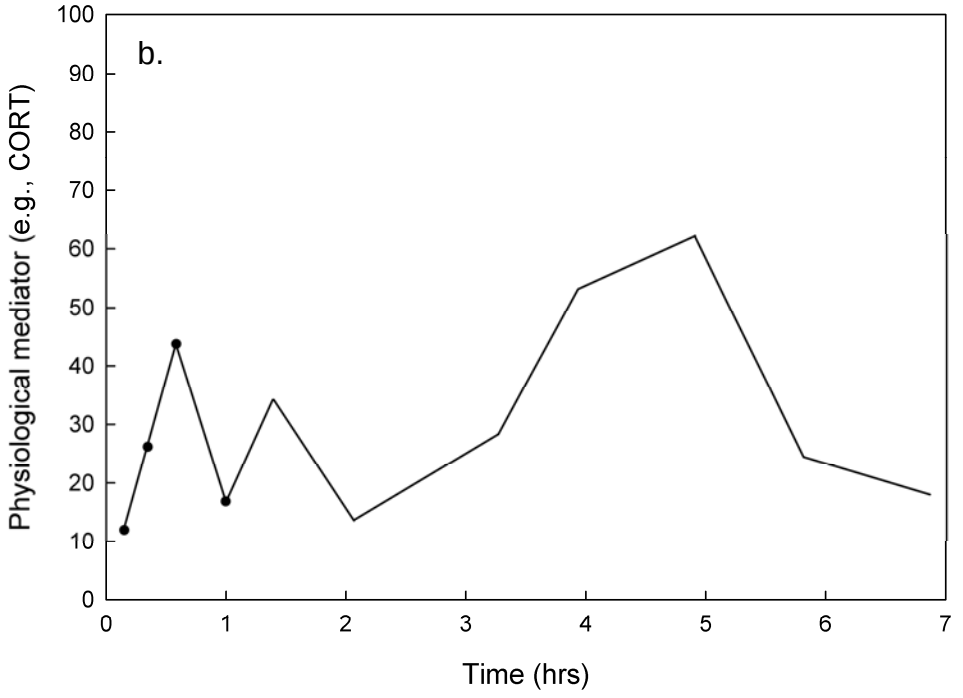
The integrated measure that fecal glucocorticoid metabolites (FGMs; [93]) provide has been used successfully, notably in conservation and captivity contexts where disturbing animals must be kept to a minimum [37,94-98]. However, FGMs cannot measure integrated GC metabolism over a timeframe greater than a few days, and limitations such as handling-induced increases in hormone levels [99], significant within-sample variation [100], potential influence of temperature [101], and a short life span in the field make interpretation difficult.

GCs can be extracted from keratin-based tissues [102-104], including feathers [105-107]. A variety of compounds, including non-polar substances, present in blood are taken up by keratinizing cells and thus deposited into feather tissue as it develops [108]. In the case of non-polar hormones, such as CORT, this is possible because they are transported by binding proteins that make them soluble in aqueous tissues such as blood [109].

Extracting CORT from feathers offers two main advantages over previous methods. First, because mature (i.e., fully grown) feathers lack a blood supply, they are not influenced by handling-induced increases in CORT. Second, feather CORT values integrate hormone secretion over the period of feather growth (Fig. 1.1; [105]), which can be days to weeks. Thus, feather CORT provides a longer-term measurement of GCs than blood or feces, and may be more biologically-relevant because it better represents the total amount

of CORT secreted over a particular period of time [89]. This perspective is better matched to understanding how GCs vary with behaviour and longer-term ecological phenomena [110]. Moreover, feathers can be used for "remote sensing" of CORT data because they may be grown in a less accessible location (e.g., breeding grounds) but collected easily in other locations (e.g., migration station).


a.
Physiological mediator (e.g., CORT)
Time (mins)
0
10
20
30
40
50
60
70
80
90
100


b.
Physiological mediator (e.g., CORT)
Time (hrs)
0
1
2
3
4
5
6
7
10
20
30
40
50
60
70
80
90
100

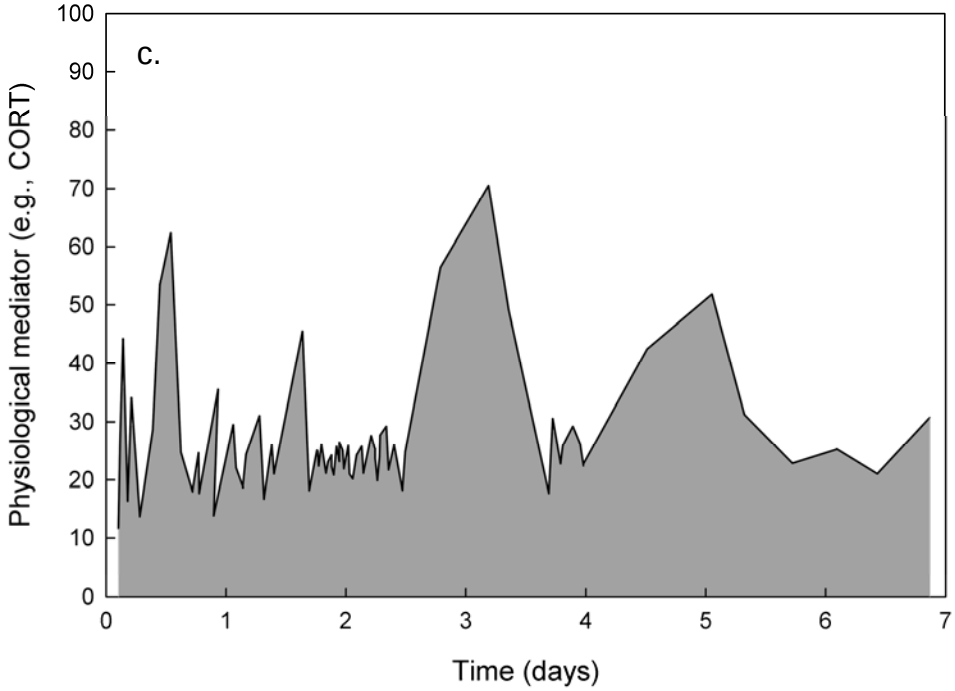


Figure 1.1. Relationship of plasma CORT measurements to an integrated measure of CORT from a feather taking approximately one week to grow. (a) A hypothetical time course of CORT derived from four CORT measurements taken using a standard capture and handling protocol. Baseline values are assumed if individual is sampled within 3 minutes and stress-induced represents the maximum value attained over sampling time (arrows). (b) Same four CORT measurements (black dots) portrayed in relation to a hypothetical 24-hr time course of CORT. (c) Same 24-hr time course in relation to a week-long period of CORT secretion captured by a feather; an integrated measure of total CORT secreted is represented by shading under the curve.

1.5 Thesis objectives

1.5.1. Overview

The objective of my thesis is to gain an understanding of the ecophysiological perspective provided by feather CORT. To accomplish this, I will 1) determine how feather CORT varies with ecological context, and 2) synthesize this information into a generalized understanding of what feather CORT values represent, how context-specific feather CORT is, and how this information can further our understanding of avian ecology. Specifically, I will address the following questions: What are the sources of environmental variability that contribute to variation in feather CORT? What role does CORT play in mediating the relationship between the individual and its environment? Can feather CORT provide information relevant to a perspective other than baseline and stress-induced?

My thesis is divided into five data chapters. These studies address feather CORT from both nestling and adult perspectives because the sources of environmental variation that influence feather CORT, and the role hormones plays in the biology of the individual, are likely to vary with age. For example, organizational effects of GCs are likely to be more influential in developing young than in adults, but the information content (i.e., sources of potential stressors) of adult environments is greater than the nest environment [7]. Additionally, the psychological experience of adults differs from that of nestlings, which likely influences responses to stressors [89]. To add breadth and additionally highlight the diversity of applications of feather CORT, I use four different study systems. Collectively, these studies consider life-history, behavioural, temporal, and spatial contexts.

1.5.2. Data chapter synopses

Quality of the rearing environment can have profound effects on the development and function of the HPA axis [82,111]. So, I investigate how nestling feather CORT relates to the physical and behavioural dimensions of early life. To gain a life-history perspective, I use two nestlings of species at opposite ends of the life-history spectrum. In Chapter 2, I address the influence of nest box microclimate on nestling tree swallows (*Tachycineta bicolor*). Nestlings were cross-fostered between two types of nest boxes that differed in microclimate. I test the hypothesis that increased variability in microclimate should promote energetically-expensive heating and cooling behaviours. I predict that feather CORT should be higher in more variable microclimates to support increased thermoregulatory behaviours. In Chapter 3, using nestling Cory's shearwaters (*Calonectris diomedea*), I investigate how individuals cope physiologically with the costs of experimentally-reduced parental provisioning. I test the hypothesis that experimental reductions in parental provisioning should be experienced by nestlings as a nutritional stressor. Based on the developmental characteristics of shearwaters, I predict that nestlings facing such stressors should have lower feather CORT than controls.

Chapters 4, 5, and 6 consider adult feather CORT at two spatial scales. In Chapter 4, I use a captive non-breeding population of wild-caught Clark's nutcrackers (*Nucifraga columbiana*) to address the influence of environmental change at a smaller, individual, scale. Although environmental enrichment is generally seen as beneficial, to the individual it may constitute an unpredictable change of environment to which the HPA axis should be sensitive. I predict that feather CORT should increase with the addition of objects to an

otherwise un-enriched environment. By considering both short- and long-term enrichment, I additionally determine the temporal context of exposure on feather CORT.

In Chapter 5, I consider adult tree swallows breeding in nest boxes. I test the hypothesis that CORT from feathers grown post-breeding reflects the energetic expense of reproduction, and predict that greater productivity will be reflected by higher feather CORT. I further predict that boxes with more stable microclimate will attract higher quality birds with higher feather CORT, because only high quality birds are predicted to be able to cope with increased levels of CORT. By addressing how CORT relates to timing of reproduction, productivity, and type of nest box used in two consecutive years, I will determine if feather CORT measures the expense of reproduction, individual quality, or both.

In Chapter 6, I consider feather CORT as a measure of habitat change at the local and landscape levels. Habitat degradation and fragmentation are important drivers of population declines, especially for grassland bird species. Habitat change may increase stressors for birds living in remnant habitat patches. Using a highly-fragmented metapopulation of the endangered Dupont's lark (*Chersophilus duponti*) as a model, I determine if synchronizing measures of feather CORT with stable isotopes (SIs) can provide information about environmental conditions (SIs) and a physiological response to those conditions. I compare how feather CORT relates to SIs and measures of habitat quality and spatial structure known to influence lark population dynamics.

Chapter 2. Postnatal corticosterone response to nest microclimate in an altricial bird[1]

2.1. Introduction

Offspring of altricial species of birds are almost completely dependent on their parents for provisioning and protection during early life. Our understanding of how altricial young respond hormonally to their environment during early life is poor, despite strong evidence of the importance of early environment on development and fitness [7,80,112]. The information content of early post-natal environments, such as within nests, is restricted relative to the external world, and this limits the information cues available to young animals [7]. Yet, individuals must respond appropriately to environmental variation to maintain homeostasis. The hypothalamic-pituitary-adrenal (HPA) axis is essential in this regard because it responds to environmental stimuli and, through the actions of glucocorticoid (GC) hormones, helps maintain homeostasis by regulating energy availability and promoting behaviors important to survival [13,15,89]. However, prolonged elevated GC secretion in young birds, such as in response to persistent stressors, can have severe costs [81,113], including permanent effects on adult behavior and HPA axis responsiveness [80,82]. By mediating the trade-off between growth and survival in developing young, GCs play an important role in shaping offspring phenotype and influencing fitness [80,82,84,114]. Identifying factors that contribute to increased GC secretion in neonates can therefore help us better understand the consequences of early-life environment to individual phenotype.

[1] I gratefully recognize the contributions of G. Treen, R.G. Clark, and G.R. Bortolotti to this work.

Much of our knowledge of the ecology of corticosterone (CORT; the primary avian GC) in birds is based on responses to a standardized capture, handling, and restraint protocol intended to mimic an acute stressor [89,115]. While this approach has yielded a wealth of information, our understanding of responses by nestlings to sources of environmental variation is still limited. This can be partly attributed to a lack of a robust response to stressors in developing altricial birds [116]. However, an immature response to stressors does not mean that we can ignore smaller increases in CORT, such as in response to less than severe (e.g., capture and handling) environmental variation. A complete understanding of how CORT influences behavior and fitness requires consideration of smaller increases in CORT such as responses to environmental variation with minimal severity [70,81]. Moreover, CORT responses to stressors may not be as important as variation in baseline levels for predicting fitness [70] or regulating predictable life-history events such as post-natal development [11].

Cumulative evidence suggests that CORT is an important physiological link between responses to the environment and phenotypic variation in neonatal birds [62,80,81,83]. However, no study has tested this by relating experimental variation in post-natal nest microclimate to CORT in altricial nestlings. Microclimate is important to the energy balance of altricial young because their ability to thermoregulate is limited [117] and can affect nestling energy budgets (see [118]), growth rates [119], and fitness [120]. The microclimate of nest cavities is more stable than that of open cup nests [121], so such cavities are ideal for studying the influence of small-scale thermal variation on CORT secretion.

Evidence from precocial birds indicates that CORT plays a role in thermoregulation [122]. As altricial birds develop the ability to thermoregulate, CORT should facilitate energetically-costly behaviors such as shivering, panting, and wing spreading because of its role in energy regulation [13,15] and respiration [123]. Furthermore, CORT can covary with other physiological systems that promote thermoregulation [124]. However, the limited research in altricial nestlings has only considered effects of weather and is correlative at best [125,126], indicating a clear need for further investigation.

To address the relationship between microclimate and nestling CORT, we cross-fostered nestling Tree Swallows (*Tachycineta bicolor*; "swallows") between two types of nest boxes that differed in microclimate: thinner-walled standard "plywood" boxes and thicker-walled "aspen" boxes made from *Populus tremuloides* logs. Importantly, we used an integrated measure of CORT from nestling feathers to quantify physiological responses. This approach avoided the potential biases and risks of blood sampling [105,127,128] and enabled us to consider how much CORT had been secreted throughout the period of feather growth. Feather CORT values incorporate the amplitude and duration of all CORT secretion, including baseline and response to stressors, during the period of feather growth [105] and thus represent a biologically-relevant measure of total CORT secretion [89].

Based on previous results from nest cavity studies [129], we predicted that the interior of plywood boxes should experience greater variability in microclimate relative to aspen boxes. Although colder ambient temperatures should promote an increase in nestling CORT to facilitate warming [122,125,126], parental brooding may at least partially buffer nestlings from the effects of cold. By contrast, in response to warmer temperatures, spatial and behavioral constraints limit cooling options available to nestlings, making

coping with warmer temperatures more problematic [120,130]. Therefore, we predicted that 1) nestlings hatched and raised in plywood boxes should have higher CORT levels to facilitate increased thermoregulation compared to nestlings hatched and raised in aspen boxes; and 2) nestling CORT would be more strongly related to maximum than minimum temperatures. Additionally, because the majority of their feather growth would take place in the foster box, we predicted that 3) nestlings cross-fostered from their natal box soon after hatching and reared in a different box type would have CORT values similar to their foster siblings.

2.2. Materials and methods

2.2.1. Field methods

Work was conducted at St. Denis National Wildlife Area, SK, Canada, from May-August 2009. We erected 25 standard and 25 aspen nest boxes in similar habitat and alternated box type to avoid clustering either box type. Neither internal box chamber dimensions nor size of entrance hole differed between box types, but wall thickness was 1 cm in plywood boxes and ~4 cm in aspen boxes. Considering the adults that raised the nestlings in our study, after controlling for sex, there was a non-significant tendency for parents in aspen boxes to be of a slightly younger minimum age (2.3 *vs.* 2.9 yrs; $F_{1,58} = 3.94$, $p > 0.05$); we detected no differences between box type in clutch initiation date, number of chicks fledged, parental body condition, or whether the individual was hatched or

previously bred in the study area, (all $p > 0.28$). We therefore concluded that parental quality was likely similar between box types.

To assess differences in microclimate we installed data loggers (HOBO Pro V2, Onset Computer Corp., USA) inside nest box chambers on the back wall (farthest from the nest hole) of nest box chambers and slightly above the nest. All data loggers were calibrated before and after being placed, and were checked for consistency among units. We alternated deployment of each data logger between an aspen and a plywood box to guard against any problem with a specific unit. Data loggers measured temperature (accuracy of ±0.2 °C from 0 to 50 °C) every minute for 27 hrs when nestlings were 8-10 days old (the period when tree swallow nestlings develop thermoregulatory ability [131]). Matched boxes (see below) were measured concurrently. Additionally, to provide context to observed patterns of feather CORT and microclimate, we determined daily maximum and minimum ambient temperatures for the period beginning when we measured microclimate and ending with the date of the final collected feathers. Ambient weather data was collected by Environment Canada at a weather station within our study area.

At 2-3 days post-hatch we cross-fostered two nestlings between plywood-aspen box pairs matched by hatch date (i.e., nestlings hatched within 24 hrs of each other; n = 18 pairs). We avoided fostering the heaviest and lightest nestlings within any brood. On day 16 post-hatch, two flank feathers were collected from two fostered and two non-fostered nestlings where possible from each box pair and stored in envelopes. All field methods complied with the University of Saskatchewan Animal Care and Supply/Ethics Board protocol #20070041.

2.2.2. Corticosterone analysis

All collected feathers were stored in plain paper envelopes until extraction. Extraction of CORT from feathers followed [105] and has been replicated with tree swallows [132] and other species [107,133,134]. We measured each feather from end to end (i.e., proximal calamus to distal tip of the vane) by flattening and straightening the entire feather length against a metal ruler. We then removed and discarded the calamus and remeasured the remaining sample feather length as above. The feather was placed in a glass vial and cut into pieces <5 mm^2 with scissors. We added 10 mL of methanol (HPLC grade, Fisher Scientific, Fairlawn, New Jersey, USA) to each sample and placed the samples in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight in a water bath. We inserted a plug of synthetic polyester fibre in a filtration funnel and separated the methanol from feather using vacuum filtration. The methanol extract was then placed in a 50 °C water bath and evaporated under a fume hood. We reconstituted dried extract residues in a small volume of phosphate buffered saline (0.05M, pH 7.6) and subsequently froze them at –20 °C until we analyzed them by radioimmunoassay (RIA). We assessed the efficiency of the methanol recovery using three additional feather samples each spiked with a small amount (approximately 5000 CPM) of ^{3}H-corticosterone in the extraction. Samples used for recovery efficiency were sonicated, incubated, and filtered as above. For additional information about extraction validation, see Supplementary Appendix S1 in [105]. Samples were extracted in two batches, with 95% and 96% of the radioactivity being recoverable from reconstituted samples, respectively. All CORT values were adjusted by recovery efficiencies.

Sample residues were reconstituted in phosphate buffered saline and analyzed by routine radioimmunoassay procedures in duplicate. We incubated 200 µL of antiserum with 100 µL of extracted samples (or standards) and 100 µL (approximately 5000 CPM) of ^{3}H-labeled corticosterone overnight (at least 16 hrs) at room temperature. Subsequently, we used a dextran-coated charcoal stripping technique to separate bound and free hormone.

Antiserum and purified CORT for standards were purchased from Sigma Chemicals, and ^{3}H CORT was purchased from Amersham Bioscience. Samples were measured in three assays with an average intra-assay coefficient of variation, calculated from three known-concentration standards, of 8.2% and an inter-assay coefficient of variation of 9.4%. Assays had a mean (± SD) limit of detection (80% bound) of 11.77 (± 1.65) pg CORT/assay tube, but data values were well above this limit and we had no undetectable samples. Because CORT deposition in feathers is time-dependent [108], data are expressed as pg CORT per mm of feather, which gives a valid estimate of CORT per unit time of feather growth [105,106]. Assays were performed at the University of Saskatchewan, Canada.

2.2.3. Statistical analyses

To characterize differences in microclimate between box types, we used four generalized linear mixed models (GLMMs) using PROC GLIMMIX in SAS v. 9.1 (SAS Institute, Cary, NC, USA) with the following response variables: mean box temperature, standard deviation of box temperature, maximum temperature, and minimum temperature. We included type of nest box as a fixed factor in all models, and the Julian

date we recorded microclimate as a continuous random factor to control for temporal effects.

We log-transformed CORT data to improve normality. To test for differences in feather CORT among experimental groups in our cross-fostering experiment, we fit GLMMs to our data following a statistical approach similar to other cross-fostering experiments (e.g., [135]). We used CORT as a response variable and included the following fixed factors: type of nest box of rearing (aspen or plywood), whether an individual was fostered or not, a box type × fostered interaction term, and calendar date of feather collection to test for possible temporal effects on CORT. We included both natal nest and nest of rearing as random variables to account for variation due to individual nests and the fact that four nestlings were sampled from each nest (i.e., clustered data). We used a stepwise elimination of non-significant terms to reach a final model containing at least box type of rearing.

To assess the overall influence of microclimate on CORT, we modeled standard deviation of box temperature, and mean, maximum, and minimum box temperature as separate fixed factors in GLMMs because these four variables were all associated with each other (PROC REG: standard deviation *vs*. mean: $r^2 = 0.25$; $p = 0.002$; standard deviation *vs*. max: $r^2 = 0.82$; $p < 0.0001$; standard deviation *vs*. min: $r^2 = 0.36$; $p = 0.0001$; maximum *vs*. minimum: $r^2 = 0.15$; $p = 0.02$). All models used CORT as the response variable, calendar date of feather collection as a fixed factor, and included natal nest and nest of rearing as random variables. We used a stepwise elimination of non-significant terms to reach a final model containing at least the microclimate variable.

2.3. Results

2.3.1. Nest box microclimate and ambient conditions

We found no significant difference between box types in mean ($F_{1,32}$ = 2.43, p = 0.13; Fig. 2.1a) or average minimum ($F_{1,32}$ = 0.62, p = 0.44; Fig. 2.1d) temperatures. Temperature variation of aspen boxes was characterized by significantly smaller standard deviations relative to plywood boxes ($F_{1,32}$ = 9.44, p = 0.004; Fig. 2.1b). Similarly, aspen boxes had lower average maximum temperatures relative to plywood boxes ($F_{1,32}$ = 23.63, p < 0.0001; Fig. 2.1c). Ambient weather data revealed that daily maximum temperatures did not exceed 30 °C (mean ± SE: 20.4 ± 0.84) and daily minimum temperatures did not drop below 3 °C (mean ± SE: 9.3 ± 0.59) during the period beginning when we measured microclimate and ending with the final date of feather collection.

2.3.2. Feather CORT

Irrespective of microclimate, fostering was not related to CORT ($F_{1,70}$ = 0.07; p = 0.77; Fig. 2.2), and there was no interaction between box type of rearing and the effect of fostering ($F_{1,70}$ = 2.64; p = 0.11). Date of feather collection was not significantly related to CORT ($F_{1,70}$ = 1.93; p = 0.17). Our final model retained only box type of rearing but this term was not significant ($F_{1,84}$ = 0.60; p = 0.44), suggesting that nestlings hatched and raised in

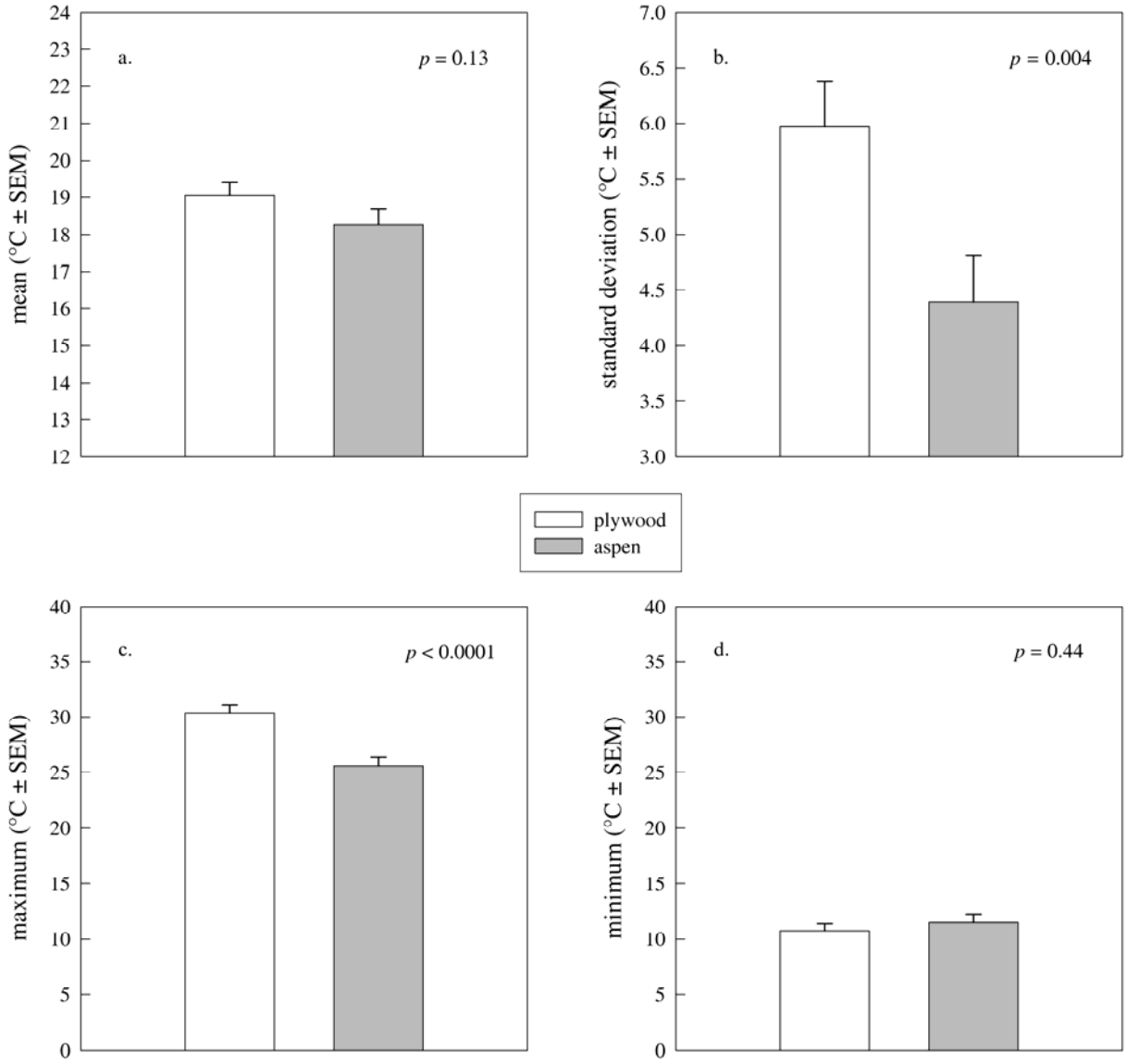


Figure 2.1. Microclimate of thinner-walled "plywood" and thicker-walled "aspen" nest boxes recorded using data loggers. (a) Mean temperature; (b) temperature variability, expressed as average standard deviation; (c) maximum temperature; and (d) minimum temperature. Significance values reported are for differences between box types.

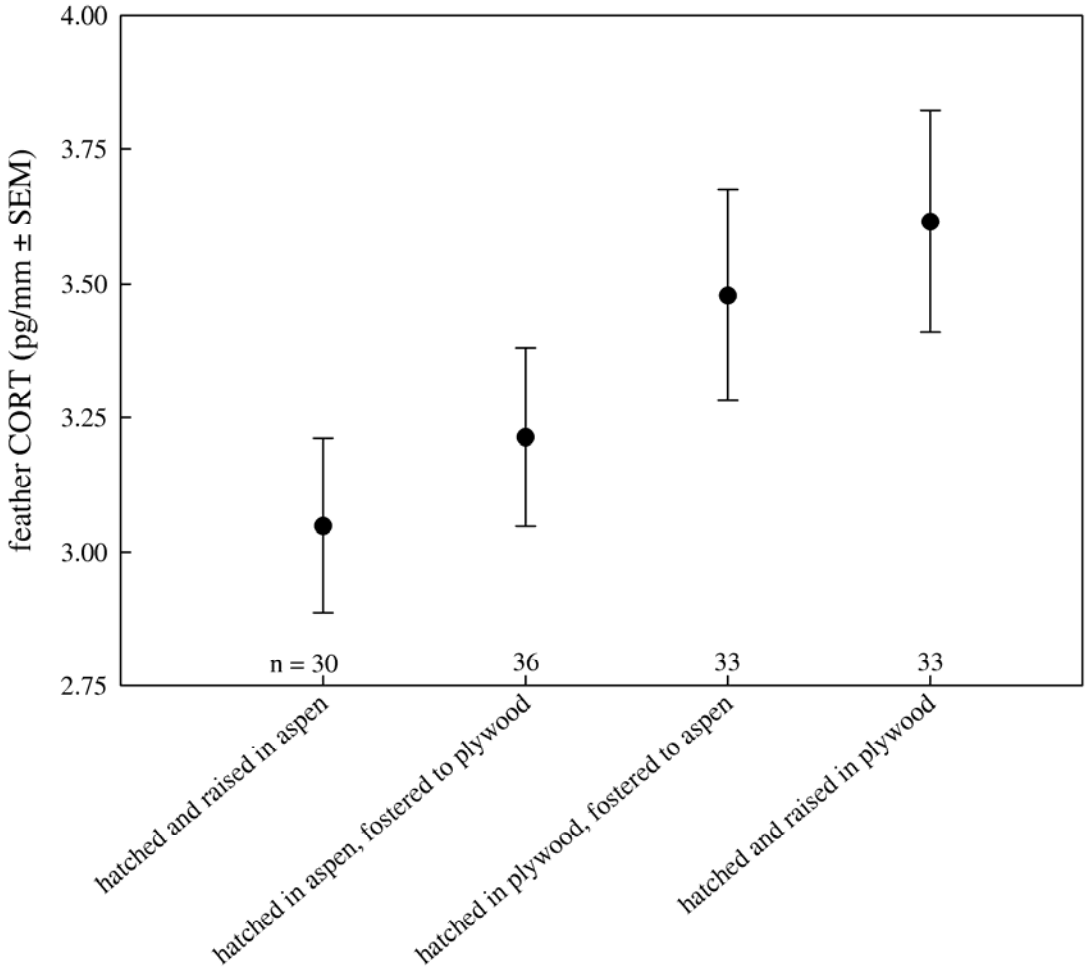


Figure 2.2. Mean (± SE) feather CORT values from nestlings in cross-fostering experiment with two types of nest boxes: thinner-walled "plywood" and thicker-walled "aspen". Individuals were hatched in plywood or aspen boxes, then either raised in their natal box or cross-fostered to a different box type.

plywood boxes did not have significantly different feather CORT values than nestlings hatched and raised in aspen boxes (Fig. 2.2). Similarly, fostered nestlings in both types of nest boxes had CORT values that were statistically indistinguishable from both their biological and foster siblings (Fig. 2.2).

Final models of the influence of microclimate on CORT revealed that, irrespective of manipulation, there were positive relationships between nestling CORT and both temperature variability ($F_{1,79}$ = 4.94; p = 0.03; Fig. 2.3a) and higher maximum temperatures ($F_{1,79}$ = 4.37; p = 0.04; Fig. 2.3b). Neither mean ($F_{1,79}$ = 1.82; p = 0.18) nor minimum ($F_{1,79}$ = 0.78; p = 0.38) temperatures were not significantly related to CORT. Date of feather collection was not significant in any of these models (all p > 0.22).

2.4. Discussion

Our results support the hypothesis that variation in CORT from nestling swallow feathers is related to nest box microclimate during the nestling period. Higher maximum, but not lower minimum, nest box temperatures were positively correlated with nestling CORT, as we predicted. Also in accordance with our predictions, microclimate differed significantly between aspen and plywood boxes. However, contrary to our expectations, the effect of microclimate on feather CORT was independent of the type of nest box, and cross-fostering appeared to have no effect on feather CORT.

Did relatively higher nestling feather CORT values reflect responses to thermal challenges? Previous studies of cavity-dwelling nestlings concluded that maximum

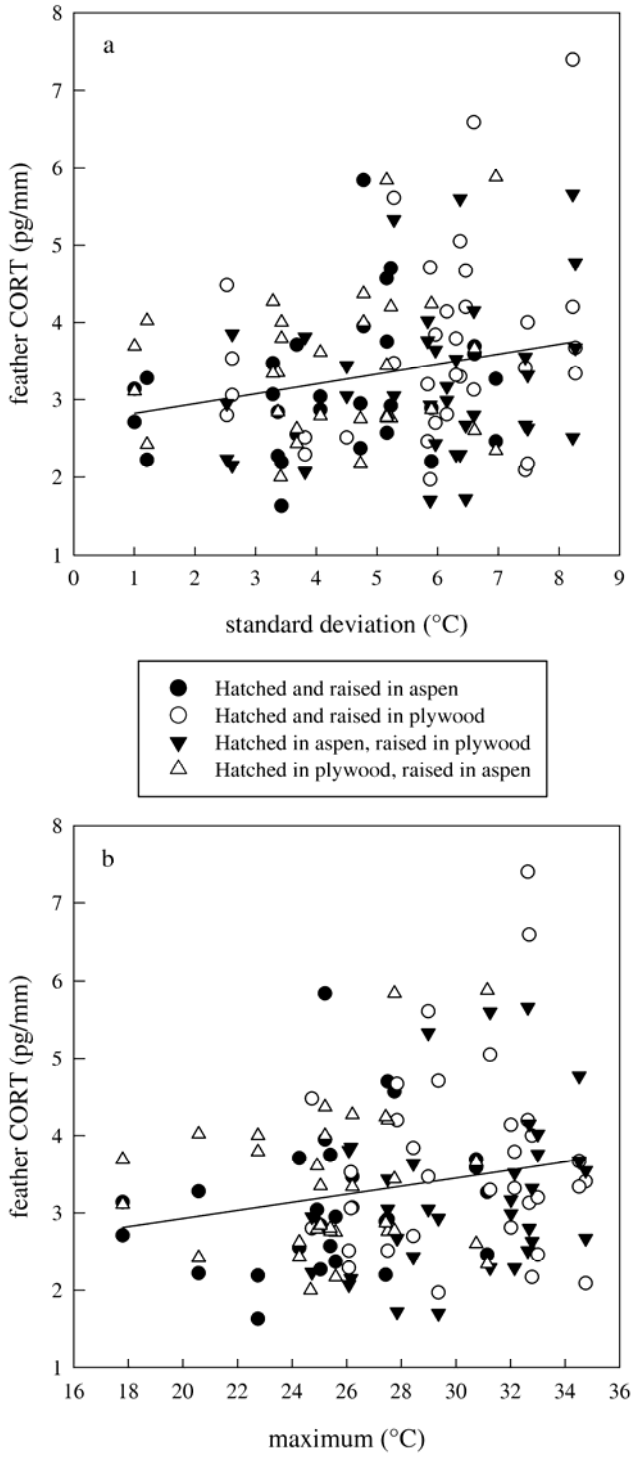


Figure 2.3. Relationships between influential microclimate variables and feather CORT of nestlings in four experimental groups. (a) Temperature variability, expressed as average standard deviation; (b) maximum temperature.

temperatures similar to those we report can be thermally challenging [120,126] and found a similar positive association between maximum (ambient) temperature and CORT [126].

During colder periods nest-bound birds can huddle to effectively conserve heat and parents compensate by brooding more [130,136,137] which reduces the amount of energy nestlings have to expend [138]. By contrast, nestling ability to dissipate heat is limited to energetically expensive cooling behaviors like wing spreading and panting [117,130,139,140], which increased CORT would facilitate [15,123]. However, even the highest nest box temperatures we measured were within the thermoneutral zone for nestling swallows [131,141], and weather data indicated that ambient temperatures throughout the rest of the feather growth period were relatively moderate. Thus, maximum temperatures within nest boxes probably were not thermally challenging to nestlings.

We therefore conclude that feather CORT values reflect individual variation in subtle energetic adjustments to thermal conditions. Nestlings expressed a range of CORT values that were within a normal range of physiological functioning (i.e., within the normal reactive scope; [142]), and individuals matched their CORT physiology to the thermal conditions within their respective nest boxes. It is additionally possible that the relationship between feather CORT maximum nest box temperatures may reflect developmental effects. Warmer nest box environments have been shown experimentally to increase growth rates and survival of nestlings, apparently through energetic savings [119,143,144]. In altricial nestlings, the HPA axis becomes more responsive as nestlings develop [116,145]. In our study, the nestling HPA axis was probably not fully developed during the period of feather growth [146]. If warmer nest box temperatures promoted nestling development, then it would follow that the HPA axis would also be maturing.

Integrated measures of CORT would therefore be higher in warmer nest box environments, reflecting greater hormonal responsiveness.

If microclimate differed between nest box types, why did we fail to detect an effect of box type on CORT? The two nest box types may not have presented significantly different biological effects to nestlings. Differences in maximum temperatures between box types were relatively small (~5 °C). Although this temperature difference can have important developmental consequences (e.g., [119]), maxima in our study did not exceed the thermoneutral zone for swallows and were likely not challenging, as we conclude above. Moreover, the relatively moderate weather in our study area suggests that temperatures extreme enough to generate biological differences between box types may not have occurred frequently during our study. Feather CORT is an integrated measure that incorporates hormonal responses to all events occurring within the feather growth period [105,133]. If temperatures high enough to present a challenge to nestlings in plywood boxes were relatively infrequent, then they may not have influenced the overall integrated measure of CORT. Thus, we conclude that the biological differences in microclimate between box types were probably not strong or frequent enough to cause between-group differences in an integrated measure of CORT. This explanation also accounts for a lack of effect of cross-fostering: nestlings were likely fostered into a microclimate that was biologically similar to their natal microclimate.

Our experimental results only partially agree with those of two previous studies that correlated nestling CORT levels with ambient weather [125,126]. Although our results are similar to those of [126], they differ from those of [125] who found that nestling CORT levels were negatively related to temperature. Mean temperatures were not significant in

our models, but when [126] considered a longer time period they also found a negative relationship with mean temperature, but only in one year. These differences between studies may, to some extent, reflect the methodology and species used in each study. We measured nestling CORT secretion in feathers, which provides a fundamentally different, and substantially longer, perspective on CORT physiology than blood or fecal sampling [105]. Also, we measured the microclimate of individual nest box cavities using data loggers, which is a different thermal perspective than ambient conditions. Nestlings on the Saskatchewan prairie likely experience a different thermal regime than nestlings in Switzerland (i.e., [125]) or central Spain (i.e., [126]). Thus, nestling CORT responses to microclimate will likely be most strongly related to local climatic factors that are most severe, and this can vary between locations, species, and years.

Our findings provide insight into the influence of environmental change on avian physiology. We present additional evidence that developing altricial birds respond hormonally to sources of environmental variation that are less than stressful (e.g., capture and handling). Our study also highlights how differences in nest environments must be considered in the context of ambient weather. Previous studies have investigated CORT as a physiological link between early life environment and a range of well-established phenotypic effects including altered growth [113], behavior [81], and CORT response [82]. Our results suggest that CORT may mediate the influence of early-life temperature on nestling phenotype. This is an important theoretical connection, considering the growing concerns over the effects of climate change on avian reproduction [e.g., 147]. Future studies should explore this possibility and determine if nestling CORT reflects subtle energetic adjustments or developmental benefits of warmer temperatures.

Chapter 3. Feather corticosterone of a nestling seabird reveals consequences of sex-specific parental investment[2]

3.1. Introduction

The trade-off between current reproductive effort and future survival and reproduction has been the subject of considerable research in life-history evolution [19]. Adults of long-lived species, such as many seabirds, are expected to favour their own condition over that of their young when faced with adverse circumstances during the breeding season [19,148,149], and offspring may therefore face costs of this parental trade-off. Although most studies on seabirds support this assertion [150-153], sex differences may exist in the extent to which males and females make the trade-off. Such differences are likely due to aspects of parental investment that differ between the sexes [154]. Male and female adult seabirds can differ in foraging strategies [155-157] ability to recover body condition [155], sensitivity to chick begging [158], and contributions to nestling diet [155,159-163]. Thus, while costs experienced by chicks are expected to vary with overall energetic demands encountered by parents, there may also be sex-specific differences in the contribution of each parent to those costs. This may be especially true when one sex is not willing or able to compensate for the other, such as during times of poor food availability when parents prioritize their own condition.

[2] The content of this chapter has been published in: *Proc R Soc B* 279, 177-184. I gratefully recognize the contributions of J. Navarro, J. González-Solís, T.A. Marchant, and G.R. Bortolotti to this work.

Relatively little attention has been paid to the adaptations of offspring facing costs of adverse parental decisions and how they may contribute to overall life-history strategies. One important mechanism for coping with environmental perturbations in general is activation of the vertebrate hypothalamic-pituitary-adrenal (HPA) axis in response to unpredictable noxious stimuli (i.e., "stressors"; [89]). The HPA axis helps vertebrates regulate energy levels through secretion of glucocorticoid (GC) hormones such as corticosterone (CORT), the primary avian GC. Nutritional challenges are known stressors characteristic of intermittent feeding of seabird chicks [34,164], and the frequency of feeding, and quality and quantity of food delivered to chicks, can influence the severity of the challenge [35,165].

Interspecific variation exists in how nestling seabirds respond with CORT to reductions in caloric intake and nutritional quality [56]. Some species increase baseline or acute stress-induced CORT secretion to promote catabolism of fat stores for increased energy availability, and to facilitate begging that encourages increased parental provisioning [34,47,56,166]. In doing so they risk reduced growth rate and immune response, depletion of lipid reserves, protein catabolism, and impaired cognition as a result of prolonged CORT secretion [13,47,56,62,89,167,168]. In other species, nestlings respond to nutritional challenges by modulating activity of the HPA axis to suppress one or more parameters of the CORT response. This has been observed as a reduction in baseline or acute stress-induced levels [35], and also as a "muting" of the response, i.e., an increase or stability in baseline with no change in stress-induced [169]. It has been proposed that this CORT suppression strategy is related to a disassociation of the nutritional state of the chick and its HPA axis [35]. Thus, although this strategy comes at a cost of a slowed growth rate,

it avoids the deleterious effects of sustained elevated CORT and allows chicks to maintain protein and fat stores [35]. Why variation in CORT responses to dietary restrictions exists is not well understood [56]; however, it is apparent that CORT physiology plays a crucial role and therefore may underlie an adaptive mechanism to cope with costs of parental trade-offs [30].

A previous study of Cory's shearwaters (*Calonectris diomedea*) by Navarro and González-Solís [153] found that when one member of a breeding pair was experimentally handicapped via increase of flying costs (i.e., breeding effort) it decreased its parental investment and passed along the cost partly to its partner, but the cost was most strongly experienced by the offspring. Handicapped adults increased the duration and distance of foraging trips resulting in longer incubation stints for their partners and less food provisioned to chicks. In turn, chicks raised by handicapped pairs were smaller, lighter, and had a lower cell-mediated immune response, and the authors suggested that poor provisioning was responsible for these effects [153]. Although foraging trip length did not differ significantly between the sexes, total mass gained while foraging was greater in males than in females [153].

Here, we suggest that nestling CORT responses to parental trade-offs can explain the effects seen in chicks from the 2007 Navarro and González-Solís paper [153], and we use an integrated measure of CORT physiology [105,106] from chick feathers collected during their experiment to explore this possibility. Feather CORT values incorporate the amplitude and duration of all CORT secretion, including response to stressors, during the period of feather growth [105,106] and thus represent a biologically relevant measure of CORT secretion [89]. We hypothesize that variation in parental investment was experienced by

nestlings as variation in a nutritional stressor to which the nestling HPA axis should be sensitive. Furthermore, sex differences in how adults traded off provisioning their young in favour of their own condition should be evident in the strength of relationships between offspring CORT and each of its parents' investment.

We tested the following three predictions. First, nestling CORT should be related to variation in duration of foraging trips and foraging efficiency of parents (i.e., rate of mass gained at sea; see below) because these are measures of parental effort that vary with increasing costs to parents [153,170]. In our population, foraging costs increase with increasing trip length [153] and, at least in other populations, longer trips result in less food being delivered per day to shearwater chicks [171]. Individual differences in foraging efficiency contribute to rules governing how parents allocate energy between themselves and their offspring [172] and thus influence the costs experienced by chicks. Second, nestling CORT should be differentially sensitive to male and female foraging efficiency, but not foraging trip duration, because the sexes differ in total mass gained at sea but not in duration of foraging trips [153]. Third, nestling CORT should be suppressed relative to controls when adult foraging costs are increased by handicapping. Suppressed HPA activity is expected to occur in nestlings of species with intermittent feeding, a prolonged nestling period to compensate for slow growth rate, and parents that are relatively insensitive to offspring demands [35,56], and Cory's shearwaters exhibit all these characteristics [153,173,174]. Our study's methodological perspective adds to a limited number of investigations into physiological adaptations of nestlings to parental reductions in food provisioning.

3.2. Materials and methods

3.2.1. Study area and field methods

For more detailed information on field methods see [153]. Briefly, the study was conducted on Gran Canaria (15°47'18''N; 27°50'41''E, Canary Islands, Spain), from April to November 2004 at a breeding colony of about 150 pairs of Cory's shearwaters. Breeding pairs were randomly assigned to the control (n=14) or experimental group (n=28) and once the female had laid her egg, one adult from every pair (50:50 male:female) in the experimental group was handicapped by clipping the tips of every primary feather to increase flying costs by 5% [153,175]. Thus, pairs from the experimental group included one handicapped bird and its unmanipulated partner. Additionally, during incubation, 19 control and 19 handicapped adults were instrumented with a 10-g geolocator (GLS units, British Antarctic Survey, Cambridge, United Kingdom) to measure foraging trip duration and foraging locations. GLS units have a photoreceptor that measures light levels every 60 s, and they record the maximum reading within each 10-min interval with reference to an internal clock-calendar. Sunset and sunrise times were estimated from thresholds in light curves; latitude was derived from day duration and longitude from the time of local midday with respect to Greenwich Mean Time and day of the year, providing 2 locations day^{-1} (one corresponding to midday and the other to midnight). The accuracy of the light-level geolocation is relatively low (average error ~186 km). However, the aim of our study was not a detailed description of the foraging trips, but a comparison of the foraging behaviour between control and handicapped birds. Any position obtained in a short period, as in the

present study, is under the same accuracy error, and to avoid potential selection biases of locations we applied a homogeneous filter based solely on a velocity index (see [153] for more details). GLS units were 1/3 the mass found to have an effect on shearwater flight performance [176], so although we cannot rule out a possible influence in our study, we believe it to be negligible and the effect balanced across treatment groups.

During incubation we studied the changes in mass in all birds by weighing all birds every 3 days until foraging trip departure, and then again upon subsequent return. Birds were weighed between 1000 and 1200 hrs using a large bag and Pesola spring balances. For those birds that we weighed 2 or 3 days before departure, we estimated the mass at departure using the last mass recorded and the proportional daily loss of mass for the appropriate sex (mean daily mass loss: males=15.38 g/day, females=14.25 g/day; calculated from incubating birds that were weighed more than once).

We sampled 28 80-day-old chicks: 10 reared by control and 18 by experimentally handicapped pairs. Chicks were ringed and weighed and their culmen, tarsus, and wing were measured with digital callipers to the nearest ±0.1mm. A single back feather was taken from each chick and stored in a paper envelope for subsequent quantification of CORT (see below). Based on exact dates of hatching, all chicks were of a comparable age when feathers were collected. All feathers were fully grown when collected, began growing when chicks were ~50 days old, and completed growth around 70 days of age. Aside from changes resulting from handicapping [153], adult feeding behavior was normal throughout the feather growth period. Adults and chicks were sexed using molecular procedures [153]. Based on observations, all chicks fledged successfully and at approximately the same time.

3.2.2. Feather CORT analysis

Feather CORT assays followed [105]. Briefly, we extracted CORT from feathers using a methanol-based technique. The length of the feather was measured, the calamus was removed and discarded, and then the sample was cut into pieces <5 mm^2 with scissors. We then added 10 mL of methanol (HPLC grade, Fisher Scientific, Fairlawn, New Jersey, USA) and placed the samples in a sonicating water bath at room temperature for 30 min, followed by incubation at 50° C overnight in a shaking water bath. The methanol was then separated from feather material by vacuum filtration, using a plug of synthetic polyester fibre in the filtration funnel. The methanol extract was placed in a 50° C water bath and subsequently evaporated in a fume hood. Extract residues were reconstituted in a small volume of phosphate buffered saline (0.05M, pH 7.6) and frozen at –20° C until analyzed by radioimmunoassay (RIA). We assessed the efficiency of the methanol extraction by including feather samples spiked with a small amount (approximately 5000 CPM) of ^3H-corticosterone in the extraction. Greater than 92% of the radioactivity was recoverable in the reconstituted samples. For more information about validation, see Supplementary Appendix S1 in [105].

Feather CORT levels were determined by RIA [177]. Measurements were performed on reconstituted methanol extracts, and samples were measured in duplicate. Samples were measured in a single assay with an intra-assay coefficient of variation of 8.7%. The assay had a detectability limit (80% bound) of 14.20 pg/assay tube, but all samples were well above this value. Data values are expressed as pg CORT per mm of feather, which gives

a valid estimate of CORT per unit time of feather growth [105,106] (and see [108] for validation). CORT assays were performed at the University of Saskatchewan, Canada.

3.2.3. Variable definitions and statistical analyses

Total foraging trip duration (TD) and foraging efficiency (FE) were defined according to [153]. TD is the total number of days between departure from the nest for foraging and subsequent return. FE is the rate of daily mass gain while foraging, calculated as total mass gained during foraging trip / trip duration. TD and FE were calculated separately for 17 males (TD_{male}, FE_{male}) and 15 females (TD_{female}, FE_{female}). In nine control breeding pairs we recorded both TD and FE for both partners. For these cases, we assessed the relative parental effort of breeding pairs by computing average TD and FE values for both partners [i.e., (male+female)/2; TD_{pair} and FE_{pair}], and assessed potential sex bias in TD and FE by computing the difference between the partners [i.e., (male−female); TD_{bias} and FE_{bias}].

Because Navarro & González-Solís only collected feathers from a subset of chicks in their 2007 paper [153], we wanted to confirm that our subset of TD and FE values were not affected by a subsampling bias. We therefore used separate models with TD and FE as the response variable, adult sex and treatment as fixed factors, and included a sex × treatment interaction term. We also tested for a chick sex difference in feather CORT, as well as a possible interaction between sex and treatment, using sex and treatment as fixed factors and a sex × treatment interaction term.

To determine the influence of within-pair variation in parental investment on chick CORT, we modeled TD_{pair}, FE_{pair}, TD_{bias}, and FE_{bias} individually as fixed factors in four separate models. To further confirm which sex's behaviour had the greater influence on chick CORT, we used the same pairs but modeled TD_{male} and TD_{female} as separate terms in the same model, rather than as within-pair averages or biases, and repeated this approach for FE.

To address the relationships between parental handicapping, TD and FE, and feather CORT, we expanded our sample size by considering all cases where we had TD and FE for at least one member of a breeding pair and feather CORT data for the chick. We used CORT as the response variable in two separate models and included treatment, adult sex, behaviour (TD or FE), and a behaviour × sex interaction term as fixed factors. Non-significant interaction terms were removed from final models. All models used a normal distribution of errors and an identity link function. Data were analyzed using PROC GENMOD in SAS v. 9.1 (SAS Institute, Cary, NC).

3.3. Results

As in the 2007 paper by Navarro & González-Solís [153], duration of adult foraging trips did not differ between the sexes for control breeding pairs ($F_{1,16}$ = 2.64, P = 0.12), but we detected a non-significant trend for trip durations of experimentally handicapped females to be longer than those of males ($F_{1,13}$ = 4.17, P > 0.06). We acknowledge this as a potential subsampling bias because the original study did not find a difference between sexes in its larger sample of experimental adults [153], but combined the sexes for all

subsequent analyses. A single CORT value was three standard deviations greater than the mean, suggesting an analytical error or an individual out of the norm for our population (e.g., an ill bird); therefore this value was excluded from analyses. There was no significant interaction between chick sex and treatment on CORT ($F_{1,23}$ = 3.13, P = 0.09), and CORT did not differ between chick sexes ($F_{1,23}$ = 1.89, P = 0.18), so they were combined for subsequent analyses.

We found no significant relationship between CORT and TD_{pair} (Fig. 3.1; $F_{1,7}$ = 1.34, P = 0.28) or FE_{pair} ($F_{1,7}$ = 0.13, P = 0.73). However, when we examined the relationships between TD_{bias} and FE_{bias} and CORT, we found a non-significant effect of TD_{bias} (Fig. 3.1; $F_{1,7}$ = 4.64, P = 0.07) but a significant effect of FE_{bias} ($F_{1,7}$ = 8.12, P < 0.03). This implies that within control breeding pairs as TD_{male} increased relative to TD_{female} chicks expressed relatively higher CORT levels, albeit not significantly; and as FE_{male} increased relative to FE_{female}, chicks expressed relatively lower CORT. This sex effect was further evident in control pairs when we included FE_{male} and FE_{female} as separate terms in the same model, because the former was significantly related to CORT ($F_{1,6}$ = 10.65, P < 0.02) whereas the latter was not ($F_{1,6}$ = 2.86, P = 0.14). A similar model for TD showed that neither TD_{male} nor TD_{female} was significantly related to CORT (TD_{male}: $F_{1,6}$ = 4.01, P = 0.09; TD_{female}: $F_{1,6}$ = 0.58, P = 0.48), but the trends were in the same direction as the FE models.

When we expanded our sample to include all cases where TD and FE were measured for at least one pair member, overall experimental chicks had significantly lower feather CORT than control chicks (Fig. 3.2; experimental = 4.45 ± 0.83 pg/mm, control = 5.46 ± 1.61 pg/mm, $F_{1,23}$ = 7.08, P = 0.01). Our model of TD and chick CORT had a significant interaction between TD and adult sex ($F_{1,27}$ = 5.12, P = 0.03), so we ran

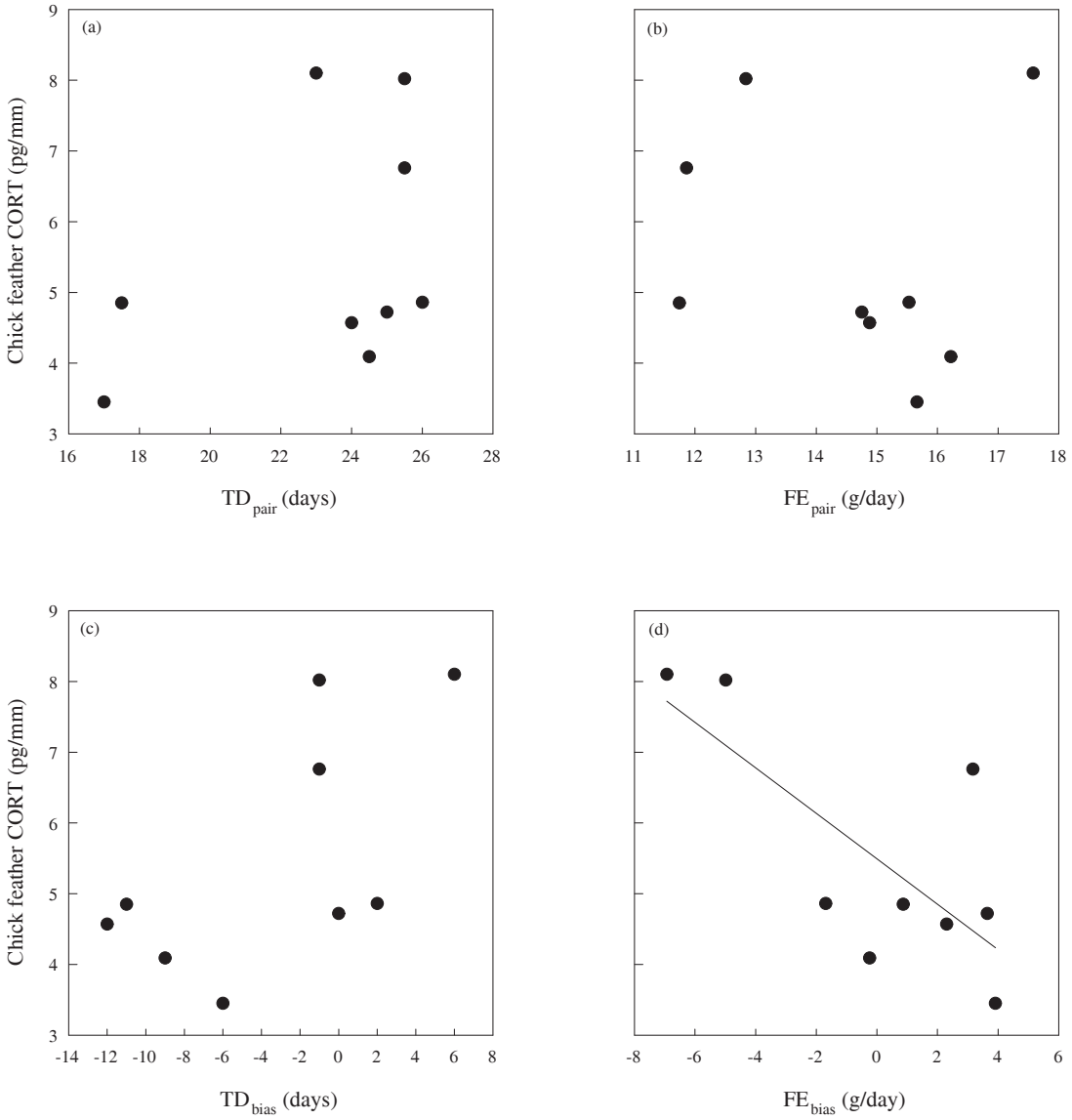


Figure 3.1. Relationships between measures of parental investment in Cory's Shearwater breeding pairs and their nestling's feather corticosterone (CORT). (a) average duration of foraging trips (TD_{pair}) and (b) average foraging efficiency (FE_{pair}). The within-pair difference between males and females in (c) duration of foraging trips (TD_{bias}) and (d) foraging efficiency (FE_{bias}); values greater than zero indicate male bias and values less than zero indicate female bias. Data presented are for control pairs only. See text for variable definitions.

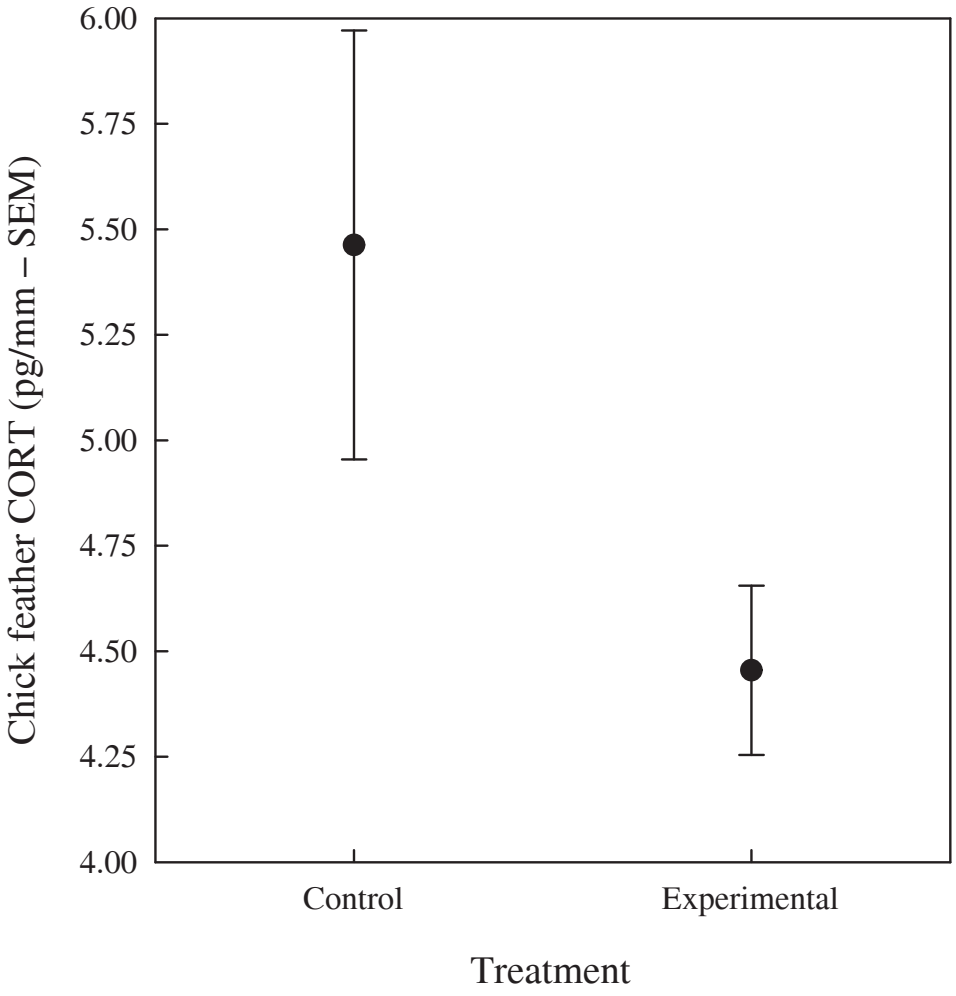


Figure 3.2. Mean (± SE) feather corticosterone (CORT) values of Cory's shearwater chicks raised by experimentally handicapped adults (Experimental; n=17) and non-handicapped control adults (Control; n=10).

separate models for each sex (Table 3.1). The final model for adult males revealed a significant positive relationship between TD_{male} and CORT (Table 3.1) and experimental chicks had significantly lower CORT than controls. The final model for adult females revealed no significant relationship between TD_{female} and CORT (Table 3.1) and experimental chicks did not differ significantly from controls.

We found a significant interaction between FE and adult sex ($F_{1,27}$ = 5.56, P < 0.03), so we analyzed the sexes separately (Table 3.1). The interaction between FE_{male} and treatment on CORT was significant, so we modeled each treatment separately for males (Table 3.1). FE_{male} was negatively related to CORT in control chicks (Table 3.1, Fig. 3.3) but was not related to CORT in experimental chicks. The interaction between FE_{female} and treatment on CORT was not significant (Table 3.1), and the final model for adult females revealed that FE_{female} was not significantly related to CORT (Table 3.1, Fig. 3.3) and did not differ between control and experimental chicks.

3.4. Discussion

Our study provides two advances in the understanding of life history trade-offs. First, we highlight the importance of sex-biased investment to offspring physiology and show that adult male shearwaters in particular play an important role in offspring energy balance. Second, we provide experimental evidence that free-living Procellariid chicks can suppress CORT secretion as an adaptive response to cope with increased costs of parental trade-offs. This result indicates flexibility in nestling physiology during growth to better match energetic need to parental provisioning. Furthermore, we show that CORT from

Table 3.1. Summary of results from GENMOD models testing for the influence of experimental handicapping of parents, sex differences in parental foraging trip duration (TD) and foraging efficiency (FE), and their interaction on feather corticosterone (CORT) in Cory's shearwater chicks. Significant values are in bold.

	Model term	estimate	standard error	*F*-statistic (df)	*p*-value
Males	Treatment	1.6053	0.6070	6.99 (1,14)	**0.019**
	TD	0.1825	0.0680	7.21 (1,14)	**0.018**
	TD × Treatment			0.00 (1,13)	0.972
Females	Treatment	0.8274	0.7968	1.08 (1,12)	0.320
	TD	-0.0958	0.0982	0.95 (1,12)	0.349
	TD × Treatment			0.02 (1,11)	0.903
Males	Treatment	7.7083	2.8695	7.22 (1,13)	**0.018**
	FE	-0.1020	0.0906	10.61 (1,13)	**0.006**
	FE × Treatment			4.91 (1,13)	**0.045**
	Control	-0.5393	0.1895	8.10 (1,7)	**0.025**
	Experimental	-0.1025	0.0809	1.60 (1,6)	0.252
Females	Treatment	0.8673	0.7479	1.34 (1,12)	0.269
	FE	0.1213	0.0949	1.64 (1,12)	0.225
	FE × Treatment			0.33 (1,11)	0.580

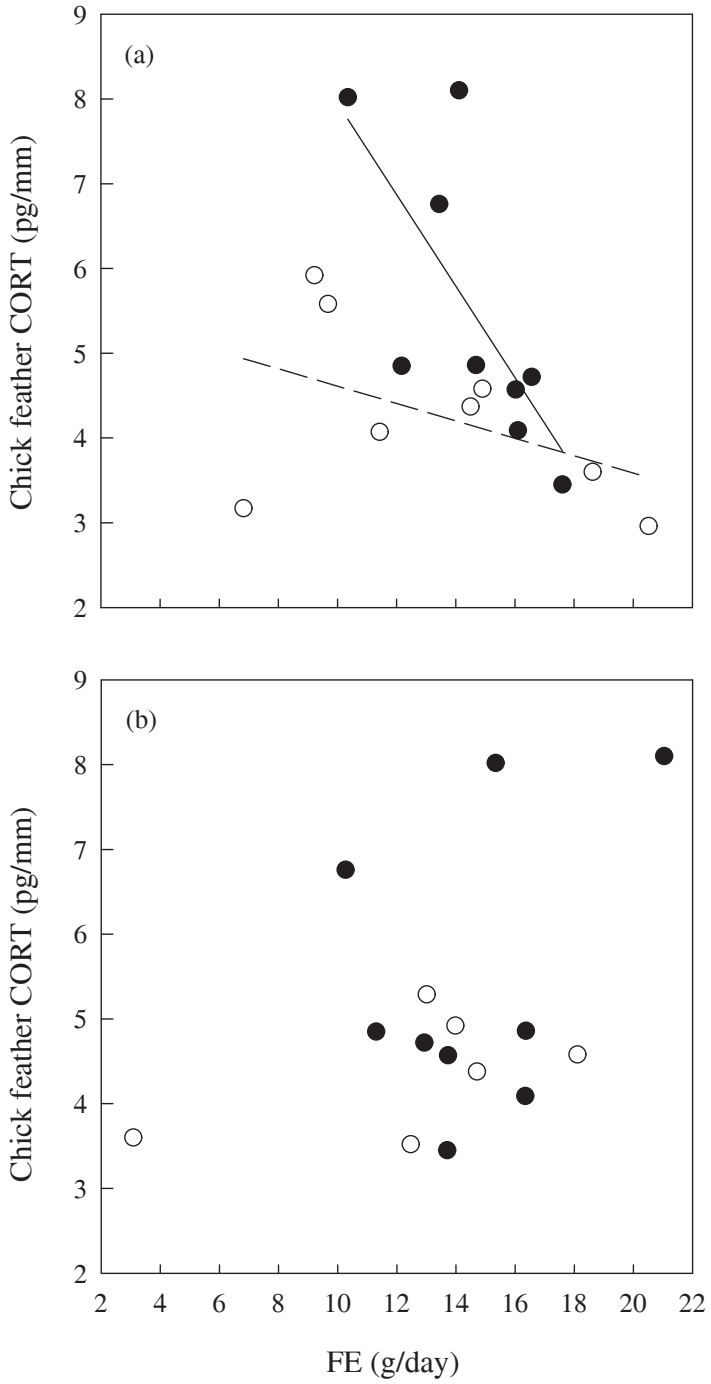


Figure 3.3. Relationships between foraging efficiency (FE) of control (filled circles, solid lines) and experimentally handicapped (open circles, dash lines) (a) male and (b) female adult Cory's shearwaters and the feather corticosterone (CORT) of their chick.

nestling feathers can provide information about how chicks are responding physiologically to variation in parental investment.

Costs are expected to arise in chicks when parents favour self maintenance over provisioning their offspring, and our study suggests that sex differences in how parents resolve this trade-off differentially affects offspring CORT. In accordance with previous studies [34,35,47,153,166,169], it is likely that an overall caloric restriction was the cost of adult trade-offs to which chick CORT was responding. Responses were related to within-pair sex biases in how parents contributed to that cost. Specifically, variation in male effort was more influential than variation in female effort.

Sex differences in parental investment in our study may have been due to differences in the extent to which the sexes were willing to increase provisioning in response to chick need [178,179]. Cory's shearwaters exhibit fixed investment in reproduction and are predicted to not increase their effort as chick demands increase [153]. However, some male Procellariiform seabirds may be even less likely than females to increase effort. For example, female Manx shearwaters (*Puffinus puffinus*) responded to chick begging by adjusting meal size, whereas males did not [158]. Additionally, during poor food years female Wilson's storm petrels (*Oceanites oceanicus*) make longer foraging trips than do males, and this may be due to greater responsiveness to chick need by females than males [180]. In cases where costs of the trade-off between self maintenance and offspring provisioning is greater in males than in females, variation in male investment could have a greater impact on chick physiology.

Importantly, we provide experimental evidence that shearwater chicks suppressed CORT secretion when faced with extended nutritional challenges. Chick CORT was most

strongly related to male foraging efficiency (FE), which is a measure of parental effort that incorporates duration of foraging trips, individual quality, and foraging decisions [172]. Not surprisingly, our results indicate that increased investment by control males reduced costs in their chicks. However, when we considered experimentally handicapped males, the CORT of their chicks showed no relationship with FE. This suggests that increased costs of trade-offs from handicapped males resulted in a relative insensitivity of the physiology of their chicks. Moreover, CORT was overall significantly lower in chicks raised by experimental parents compared to controls. We interpret these results as confirmation of our prediction that shearwater chicks suppress CORT secretion when adult foraging costs are experimentally increased.

Is lower CORT in experimental chicks a result of an adaptive response, or simply an expression of poor physiological functioning of birds with extended nutritional deficits? It is possible that the nutritional condition of experimental chicks was such that they were only able to mount a poor CORT response following nutritional challenges, or they were developmentally delayed and incapable of mounting a better response. However, it is unlikely that chicks expressing such comprised physiology would be able to survive to fledging without indicators of lipid or protein reserves, or muscle damage being affected [181]. Yet, in their 2007 paper Navarro and González-Solís found that levels of these biochemical parameters were similar between control and experimental chicks [153], and all chicks fledged at approximately the same time. Moreover, there was some overlap in the range of CORT values for each group. These data suggest that the physiology of experimental chicks was operating within normal limits. We therefore lack the evidence to support a conclusion that experimental chicks were physiologically impaired.

To the contrary, we reason that experimental chicks were within their physiological ability to handle periods of nutritional deficit. CORT suppression was therefore likely an adaptive response to cope with the increased costs of parental trade-offs. We argue that cumulative costs of parental trade-offs in experimental chicks reached a tipping point and CORT suppression allowed these birds to minimize the extent of physiological damage caused by chronically elevated CORT [13,34,56,89,164,167]. Experimental chicks paid for this because they were smaller, lighter, and had reduced immune response [153]. Yet, these were not life-threatening energy deficits because the prolonged period of shearwater nestling growth would allow for compensatory growth [35,56,153,173,174] and survival to fledging did not differ between treatment groups [153]. CORT suppression need not entail a complete alteration of the functioning of the HPA axis, as evidence from other species indicates that even the most food-restricted individuals exhibiting CORT suppression are still able to respond to stressors [35,169].

Understanding how and why individuals manage their exposure to CORT during critical periods of post-natal development is important because CORT can affect nestling phenotype [7,56,63,83,182,183] and potentially fitness [84] (for reviews see [70,87]). Moreover, timing of CORT exposure during development is important [7]. In our study, handicapping of adults occurred at the onset of egg-laying and therefore increased costs were experienced by nestlings throughout their post-natal development. Whether shearwater nestlings would suppress CORT in response to less severe or shorter-term increases in costs remains to be determined. Future investigations should focus on identifying the ecological circumstances that promote a CORT suppression strategy and must consider phylogeny, mode of nestling development (see [184]), and the type of

nutritional challenge facing nestlings (i.e., feeding frequency, diet quality and/or quantity). Longitudinal studies will be essential in identifying potential long-term costs and benefits of CORT suppression.

Chapter 4. Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective[3]

4.1. Introduction

Enrichment is the modification of a captive animal's environment with the goals of increasing environmental complexity [185] and improving biological functioning [186]. The majority of enrichment research has focused on combating fearfulness and harmful abnormal and stereotypic behaviours arising in captive production (i.e., farm), laboratory, and companion animals ([187]; see also [188]) because animal welfare is both economically and ethically important [185,189-191]. Behavioural ecologists have indirectly studied enrichment in different contexts, and more frequently use non-domesticated animals as models. Investigations of, for example, exploration behaviour [192,193] information acquisition [194], dominance [195], and personality [196,197] can involve *de facto* enrichment and provide data comparable to studies of other captive animals. Enrichment has numerous behavioural effects (for reviews see [190,198,199]), but has been shown to reduce fear responses [185,197,200], increase movement and activity [201-203], and induce changes in exploration behaviour [192,194,195,204,205].

Studies assessing physiological responses to enrichment frequently measure levels of glucocorticoid (GC) hormones like corticosterone (CORT) or cortisol because they vary with exposure to environmental perturbations [13,70,89,206]. Prolonged activation of the HPA axis and sustained elevated levels of GCs have detrimental effects on health and

[3] The content of this chapter has been published in: *PLoS ONE* 6, e17663. I gratefully recognize the contributions of M.D. Frey, J.F. Reichert, I. Szelest, D.M. Kelly, and G.R. Bortolotti to this work.

reproduction [207,208] and GC levels can correlate with fitness components [10,70,84,87]. However, while some studies have reported that enrichment can lower GC levels in the blood [209,210], others have reported no effect [203,204,211,212], or even increases [205,213,214]. Such studies have been inconsistent in their procedures for measuring GC physiology and there is still a lack of consensus and understanding of the effects of enrichment on GCs [215,216]. Furthermore, studies addressing simultaneous behavioural and physiological responses to enrichment have found that animals may react behaviourally in measurable ways yet exhibit no measurable GC response. For example, garden warblers (*Sylvia borin*) exposed to a toy exhibited active exploration [204] and great tits (*Parus major*) exposed to a box showed increased activity [203], yet neither showed a change in circulating CORT levels. Likewise, steers (*Bos primigenius*) given a drum can [217] and calves given toys [218] significantly increased active behaviours, yet showed no change in cortisol levels. How abnormal behaviours relate to stressors is unclear. For example, Dybkjaer [219] reported that belly-nosing behaviour in pigs is an indicator of stressful rearing conditions, but when Gardner and colleagues [220] manipulated pig density as a means of lowering stress, they did not detect a change in that behaviour. Furthermore, Le Maho and colleagues [221] found that although domestic geese appeared calm and exhibited no behavioural signs of stress during a routine procedure to which they had been adjusted, several-fold increases in CORT levels were detected following the procedure. This collective evidence suggests that behaviour and stress physiology are context dependent and may operate independently of each other.

From the perspective of the animal, enrichment constitutes an unpredictable environmental change. Thus, an animal's response to enrichment may not be caused by the

enrichment objects *per se*, but rather by the associated change. Although some behavioural responses to enrichment, such as exploration and play, can be attributed to the objects themselves, physiological responses may more likely be caused by the unpredictable nature of the change in environment. Vertebrates are well known to respond physiologically to such change by releasing GCs as part of the "stress response" [10,13,89].

While the vast majority of research has addressed the effects of enrichment, relatively little is known about how animals respond to a change from an enriched to a more impoverished environment. This is an important knowledge gap because it is an animal's response to removal of enrichment objects that would shed light on the importance of associated environmental change. A barren environment can affect behaviour [222] and physiology [223], and sparse evidence suggests that removal of enrichment can have negative physiological [209,217] and psychological [224] effects. However, studies experimentally testing the relationship between behavioural and GC responses to enrichment and its removal within the same animal are rare, especially for non-domesticated birds [187].

It is important to determine how well a change in behaviour correlates with measures of physiological stress [225], especially if behavioural responses to enrichment are to be integrated effectively into measures of emotional state and, subsequently, well-being and quality of life of captive animals (see [191,224,226]). Enrichment is used as a stress-reduction technique ([227] and references therein; [228]) and stress is believed to mediate the relationship between problem behaviours and well-being [229-231]. However, inconsistencies in the literature make it necessary to clarify how and when the HPA axis responds to enrichment. Better understanding the relationships between enrichment,

behaviour, and stress will help refine techniques for assessing the outcomes of enrichment procedures [231], which will benefit a broad spectrum of research.

Although the lack of consensus regarding enrichment and GC levels may be partly context dependent (e.g., different enrichment protocols; [215]), all previous studies measuring GC levels have utilized blood or, less frequently, fecal [93,232,233] or salivary [234] sampling. These techniques have known limitations and biases ([100,115]; and see [105]) and provide measures of GC physiology over short time periods (i.e., minutes or hours). Thus, our understanding of how enrichment affects stress physiology would benefit from a long-term perspective on GC secretion. Here we use a technique to track stress physiology of birds through changes in CORT found in feathers. Feather CORT integrates the intensity and frequency of the physiological response because values incorporate the amplitude and duration of all CORT secretion, including response to stressors, during the period of feather growth [105,106]. Therefore, feather CORT does not rely solely on baseline or stress-induced values, but instead integrates the two into a biologically-relevant measure of total CORT secretion (*sensu* [89]).

We conducted two experiments to help clarify the relationships between enrichment and its removal, GC physiology, and behaviour. Although domestic chickens (*Gallus gallus*) are the typical avian model for enrichment research, we wanted the results of our study to also be applicable to behavioural ecologists, so we used a captive population of wild-caught Clark's nutcrackers (*Nucifraga columbiana*). During experiment 1 we exposed nutcrackers to short-term (10-d) enrichment to test the hypothesis that enrichment attenuates stress physiology. If this were true, nutcracker feather CORT should be significantly reduced following short-term enrichment. Alternatively, if short-term

enrichment does not affect nutcracker stress physiology, or if nutcrackers perceive enrichment as a stressor, we predict no effect or an increase in feather CORT, respectively. In experiment 2 we exposed nutcrackers to enrichment objects continuously for three months, then removed the objects. This design allowed us to replicate experiment 1, using both short- and long-term enrichment, and also test the hypothesis that the change of environment associated with enrichment and its removal is perceived as a stressor. If the environmental change were a stressor, feather CORT should increase immediately following both addition and removal of enrichment objects. Additionally, we were interested in how well behavioural measures can be used as a proxy for physiological responses to enrichment, so in experiment 2 we examined the relationships between feather CORT and fearfulness, activity, and exploration behaviours.

4.2. Materials and methods

4.2.1. Ethics Statement

All aspects of this research complied fully with the rules and regulations governing the use and care of animals in research at the University of Saskatchewan, and were conducted under approval #20040088 from the Animal Research Ethics Board, University of Saskatchewan.

4.2.2. Housing and daily routine

During 2000-2002, 41 wild nutcrackers were caught in Colorado, USA, so all birds had been in captivity for at least 4 years prior to our first experiment in 2007. All birds were housed individually at the Western College of Veterinary Medicine, University of Saskatchewan, Canada, in a single windowless colony room in standard metal pet bird cages constructed from thin (~3mm) metal bars with a removable metal floor tray (1 m x 0.75 m x 1 m). All cages had a wooden perch and separate wood and metal swing. All birds were checked regularly by veterinarians and were deemed in good health before we began our experiments. Prior to experiments, all birds had experienced the same daily cleaning and feeding routine that we continued for the duration of the experiments: morning weighing, feeding, and water changing; afternoon water changing; weekly cage changes; and additional twice weekly cage bottom cleaning. Nutcrackers were fed a 95% *ad libitum* diet of turkey starter, parrot pellets, sunflower seeds, peanuts, pine nuts, mealworms, and vitamin supplement, as well as water and grit *ad libitum*. Food and grit was provided in plastic food cups snapped into the cage walls, and water was provided in circular plastic bowls. Cages were arranged on moveable racks that could accommodate three cages above and three cages below. Light was maintained at 12 h light:12 h dark. None of the birds in our experiments had previously received any form of cage enrichment other than their perch and swing.

4.2.3. Experiment 1

Beginning in October 2007, 16 randomly selected nutcrackers (8 male, 8 female) were moved from the colony room into a similar windowless experimental room and

assigned randomly to one of two walls that faced each other. After 2 weeks, a plastic curtain was installed that divided the experimental room in half: eight birds on one side of the divider were visually isolated from eight birds on the other side of the divider. Birds were allowed to adjust to the divider for an additional 2 weeks (Fig. 4.1).

We then pulled the right secondary feather #1 (adjacent to primary #1) from each nutcracker to induce new feather growth. All feathers pulled were fully grown and dead. Subsequent feather growth was measured every 5 days for the remainder of the experiment. After the first 10 days of feather growth enrichment objects to which the birds were naïve were installed in the cages of birds on one side of the divider only, thus separating birds into an enriched experimental group (n=8) and a non-enriched control group (n=8). Enrichment objects comprised three plastic bird toys (balls: 195 mm x 45 mm, rings: 190 mm x 50 mm, and a mirror lantern: 140 mm x 35 mm) and one wooden chew toy (250 mm x 55 mm) that all hung inside the cages, plus an artificial pine garland (300 mm x 300 mm) installed on the outside of the upper left back corner of the cage. All objects were added at the same time. After 10 days of enrichment, objects were removed and the regrowing feathers were allowed to grow for an additional 10 days, at which point they were pulled out (Fig. 4.1).

We chose to group all experimental birds together on one side of the room rather than randomly assign treatments to birds. Random assignment would have resulted in experimental birds being neighbors with non-experimental control birds and to reduce obvious bias we would have been forced to visually isolate neighbors. We chose to not do this for two reasons. First, it would have been difficult to keep control birds from seeing enrichment objects during cage changes. Second, and more importantly, little is known

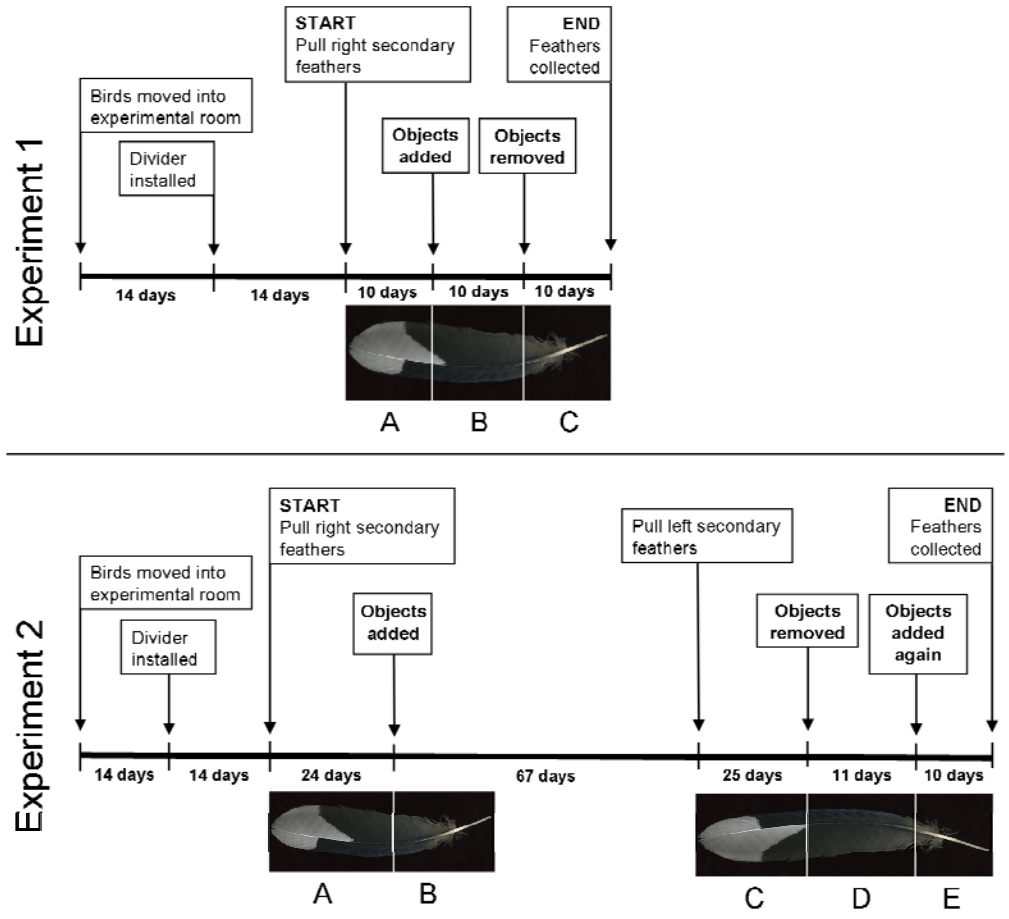


Figure 4.1. Experimental timelines with diagrams illustrating how feather sections reflect periods of the experiments. Feathers from experiment 1 were cut into three sections corresponding to periods prior to pre-enrichment (A), short-term enrichment (B), and removal of enrichment (C). The first feather from experiment 2 was cut into two sections corresponding to pre-enrichment (A) and short-term enrichment (B), and the second feather from experiment 2 was cut into three sections corresponding to long-term enrichment (C), removal of enrichment (D), and non-novel enrichment

about nutcracker sociality, but it is suggested that they are moderately social birds [235]. Birds in our experiments had always been maintained in a colony setting, so rather than confound our data by subjecting birds to a potentially stressful social situation, we chose to group birds by treatment, thus allowing individuals within a treatment group to see each other.

4.2.4. Experiment 2

Beginning in October 2008, 23 nutcrackers (12 m, 11 f) that had not previously received cage enrichment were selected randomly, moved into the experimental room, and assigned randomly to one of two walls that faced each other. The birds' daily routine was maintained throughout the experiment and was the same as that described above. We repeated the adjustment procedure as in experiment 1, except that the divider separated nutcrackers into groups of 11 and 12. We then pulled a feather from each bird to induce new feather growth (Fig. 4.1). All feathers pulled were fully grown and dead. We pulled the right secondary feather #1 from 17 of the 23 birds, but six birds were missing or growing that feather so we selected the next morphologically similar feather in sequence: right secondary feather #2 (4 birds) or #3 (1 bird), or right primary feather #1 (1 bird).

Regrowing feathers were measured every 5 days until feathers were approximately half-grown (mean ± SD = 61.74 ± 8.95 mm). Then, as in experiment 1, enrichment objects to which the birds were naïve were installed in the cages of birds on one side of the divider only, forming an enriched group (n=11) and a non-enriched control group (n=12). All objects were added at the same time. The induced feathers were allowed to complete their

growth and were subsequently pulled 31 days later to ensure that feathers were fully grown (Fig. 4.1).

Once birds had been exposed to enrichment objects for 67 days, we pulled a second feather from each individual to induce new feather growth (Fig. 4.1). All feathers pulled were fully grown and dead. We pulled left secondary #1 from 17 birds, but three birds were missing or growing that feather. For those individuals, we selected the next morphologically similar feather in sequence: left secondary feather #4 (1 bird), or right secondary feather #1 (2 birds) or #2 (1 bird). Feathers were allowed to grow for 25 days, at which point the enrichment objects were removed from cages. After 11 days the enrichment objects were re-installed into the same cages as before and feathers were allowed to grow for a final 10 days before being collected for analysis. We were only able to sample second feathers from 21 birds because two birds died and, in a third, an induced feather did not regrow.

4.2.5. Feather sections

We cut all feathers using growth measurements as guides such that cut sections corresponded to the different time periods of the experiment (Fig. 4.1). Feathers from experiment 1 were cut into three sections: the distal section was grown prior to enrichment (Fig. 4.1, top, A: “pre-enrichment”), the middle section was grown while enrichment objects were present in the cages (Fig. 4.1, top, B: “short-term enrichment”), and the proximal section was grown after objects were removed (Fig. 4.1, top, C: “removal of enrichment”). The first feathers from experiment 2 were cut into two sections: the distal

section was grown prior to enrichment (Fig. 4.1, bottom, A: “pre-enrichment”) and the proximal section was grown while enrichment objects were present in the cages (Fig. 4.1, bottom, B: “short-term enrichment”). The second feathers from experiment 2 were cut into three sections: the distal section was grown while enrichment objects were still present in the cages (Fig. 4.1, bottom, C: “long-term enrichment”), the middle section was grown after objects were removed (Fig. 4.1, bottom, D: “removal of enrichment”), and the proximal section was grown when objects were re-installed (Fig. 4.1, bottom, E: “non-novel enrichment”).

4.2.6. Behaviour

We recorded nutcracker behaviour during experiment 2 using a small digital video camera that was able to record all birds on one side of the room simultaneously (Table 4.1). The camcorder was mounted on a small tripod that stood on a utility cart that was used for daily feeding and was therefore familiar to the birds. We used the digital timestamp on the recordings for calculating elapsed time.

We measured latency to feed (LTF) for each nutcracker as the time taken by a bird to approach its food dish after being reintroduced into the cage. Previous studies have used latency to feed as a measure of fearfulness [197,236-238]. Our nutcrackers normally feed readily in the presence of caretakers, and even jump on food cups and begin to feed before the cups are fully snapped into place on the cage. Thus, we knew *a priori* that the birds should not be afraid of caretakers or food during feeding. We made 5-minute video recordings of both experimental and control nutcrackers during normal feeding time

Table 4.1. Observation schedule for nutcracker behaviors recorded during Experiment 2. LTF=latency to feed, AL=activity level, EXP=exploration. See text for definitions of behaviors. [1]All behaviors were measured for 5 mins.

Experimental period	Time of day (hrs)	Behavior[1]		
		LTF	**AL**	**EXP**
Pre-enrichment	1700		**x**	**x**
Short-term enrichment	1100	**x**		
	1700		**x**	**x**
Long-term enrichment	1100	**x**		
	1700		**x**	**x**
Removal of enrichment	1100	**x**		
	1700		**x**	**x**

(~11:00 hrs) on three separate occasions as they were released into fresh cages that already had food in cups. The first recording was made immediately following the installation of enrichment objects into experimental cages. This recording captured the initial behavioural responses of all birds at the time when experimental individuals were first exposed to enrichment objects. The second and third recordings were made when birds had received 2 months of continuous exposure to objects ("long-term enrichment"), and immediately following removal of enrichment objects 26 days later ("removal of enrichment").

Birds are known to express behaviours ranging from freezing to active investigation when exposed to novelty [237]. We therefore measured two behaviours likely to vary with exposure to novel objects: activity level (AL), quantified by counts of all hops around the cage and positional changes (i.e., turning around 180° but remaining in the same place when perched); and exploration (EXP), quantified by counting the number of pecks at any object within the cage or at any part of the cage itself. Recordings of AL and EXP lasted 5 minutes, as in previous work (e.g., [209]), and were made several hours after afternoon water changes (at ~1700 hrs) and in the absence of caretakers to ensure that these behaviours were not affected by human presence. Observations of AL and EXP were made during four periods of our experiment: 2 weeks prior to enrichment ("pre-enrichment"), immediately following the installation of enrichment objects ("short-term enrichment"), after birds had 2 months of continuous exposure to objects ("long-term enrichment"), and immediately following removal of enrichment objects 26 days later ("removal of enrichment").

4.2.7. Feather CORT assays

We extracted CORT from feathers in three separate extractions using a methanol-based technique following [105]. We measured the lengths of all feathers, and then cut, removed, and discarded the calamus. The remaining feather sample was cut with scissors into very small pieces (<5 mm^2) and 10 mL of methanol (HPLC grade, VWR International, Mississauga, Ontario, Canada) was added. We placed samples in a sonicating water bath at room temperature for 30 min, then incubated them at 50 °C overnight in a water bath. We separated the methanol from the feather material by vacuum filtration, and the methanol extract was placed in a 50 °C water bath and allowed to evaporate in a fume hood. Extracts were later reconstituted in a small volume of phosphate buffer system (PBS; 0.05M, pH 7.6) and frozen at –20 °C until analyzed by radioimmunoassay (RIA). We assessed the efficiency of each of the three methanol extractions by including feather samples spiked with a small amount (approximately 5000 CPM) of ^{3}H-corticosterone in each extraction (see Appendix S1 in [105] for more details). On average, greater than 95% of the radioactivity was recoverable in the reconstituted samples.

Feather CORT levels were determined by RIA as in previous studies [64,105,106,132,177]. Measurements were performed on reconstituted methanol extracts and were duplicated. Samples were measured in four assays with an intra-assay coefficient of variation of 7.4%, an inter-assay coefficient of variation of 14.1%, and mean (± SD) limit of detection (ED80) of 12.9 ± 2.2 pg CORT/assay tube. Data values are expressed as pg CORT per mm of feather, which gives a valid estimate of CORT per unit time of feather

growth (see [105,106,108] for validation). CORT assays were performed at the University of Saskatchewan, Canada.

4.2.8. Statistical analyses

To determine if feather sections used for CORT analyses differed in length between enriched and non-enriched controls we combined all data from both experiments and used a mixed model (PROC MIXED; SAS v. 9.1). We used length of feather section as the response variable, treatment as the explanatory variable, and experiment (i.e., 1 or 2) as a random factor. We included a repeated statement to account for multiple measurements taken from the same individual over time [239].

We used mixed modeling (PROC MIXED) to compare CORT values from feather sections grown during the periods of our experiments. For experiment 1 we compared pre-, short-term, and removal of enrichment. For experiment 2 we compared pre-, short-term, and long-term enrichment; removal of enrichment; and non-novel enrichment. We modeled feather CORT as the response variable, time period and treatment (i.e., experimental or control) as fixed factors, and included a time period × treatment interaction term. We used a repeated statement to account for multiple measurements taken from the same individual over time. Two feathers were collected from each bird in experiment 2, so our model included a random factor to account for possible variation between feathers.

To determine the influence of enrichment on behaviour, and to address the relationship between behaviour and CORT, we used mixed models (PROC MIXED and PROC

GLIMMIX; SAS v. 9.1). We modeled behaviours individually, using behavioural data (LTF or AL) as the response variable, feather CORT as a covariate, and time period, treatment (i.e., control or experimental), and a time period × treatment interaction term as fixed factors. LTF data were fitted to models using a normal error distribution and an identity link function in PROC MIXED. AL data were counts and were therefore fitted to models using a negative binomial error distribution and a log link function in PROC GLIMMIX. We used random statements to account for variation between feathers, and used repeated (PROC MIXED) or random (PROC GLIMMIX) statements to account for the multiple measurements taken from the same individual over time.

EXP data were counts, but were zero-inflated and we were not able to get models to converge using PROC MIXED or PROC GLIMMIX. Instead, we used a zero-inflated Poisson model (PROC GENMOD) to address the relationship between EXP and CORT. We used counts of pecks as the response variable, CORT as a covariate, and time period and treatment (i.e., enriched or control) as explanatory variables. Although this approach had the advantage of accounting for the high incidences of zeros in our data, it did not allow us to include an interaction term or random or repeated statements. However, considering how few counts of pecks were actually recorded throughout the experiment, we do not believe the absence of interaction and random terms affected our results significantly.

4.3. Results

Lengths of feather sections used for CORT analyses did not differ between control and experimental groups in any time period of either experiment ($F_{1,142}$=0.09, p=0.77).

4.3.1. Experiment 1

Overall, mean CORT values differed significantly between time periods ($F_{2,22}$=13.76, p<0.001; Fig. 4.2), but not between treatment and control birds ($F_{1,12}$=3.96, p=0.07), and the time period × treatment interaction was not significant in our model ($F_{2,22}$=2.76, p=0.09). However, *post hoc* comparisons revealed that CORT levels increased significantly from pre-enrichment to short-term enrichment in experimental birds ($t_{1,22}$=-4.66, p<0.001), but not in controls ($t_{1,22}$=-1.57, p=0.13). CORT values from the enrichment removal period did not differ significantly from pre- enrichment values for either group (experimental: $t_{1,22}$=-1.47, p=0.16; control: $t_{1,22}$=-1.92, p=0.07).

4.3.2. Experiment 2

Similar to experiment 1, mean CORT values differed significantly between time periods ($F_{4,93}$=16.48; p<0.0001; Fig. 4.3), but not between treatment and control ($F_{1,93}$= 0.07; p=0.79), and there was no significant interaction between treatment and time period ($F_{4,93}$=1.92, p=0.20). CORT increased significantly between pre-enrichment and short-term enrichment periods for both experimental ($t_{1,93}$=-3.82, p=0.0002) and control birds ($t_{1,93}$=-4.71, p<0.0001). Long-term enrichment CORT values in controls were similar to pre-enrichment values ($t_{1,93}$=0.03, p=0.98), but in experimental birds long-term enrichment CORT values were significantly lower than pre-enrichment values ($t_{1,93}$=3.16, p=0.002). CORT levels from feather sections grown after removal of enrichment objects increased significantly from long-term enrichment levels in experimental birds ($t_{1,93}$=-2.63, p<0.01)

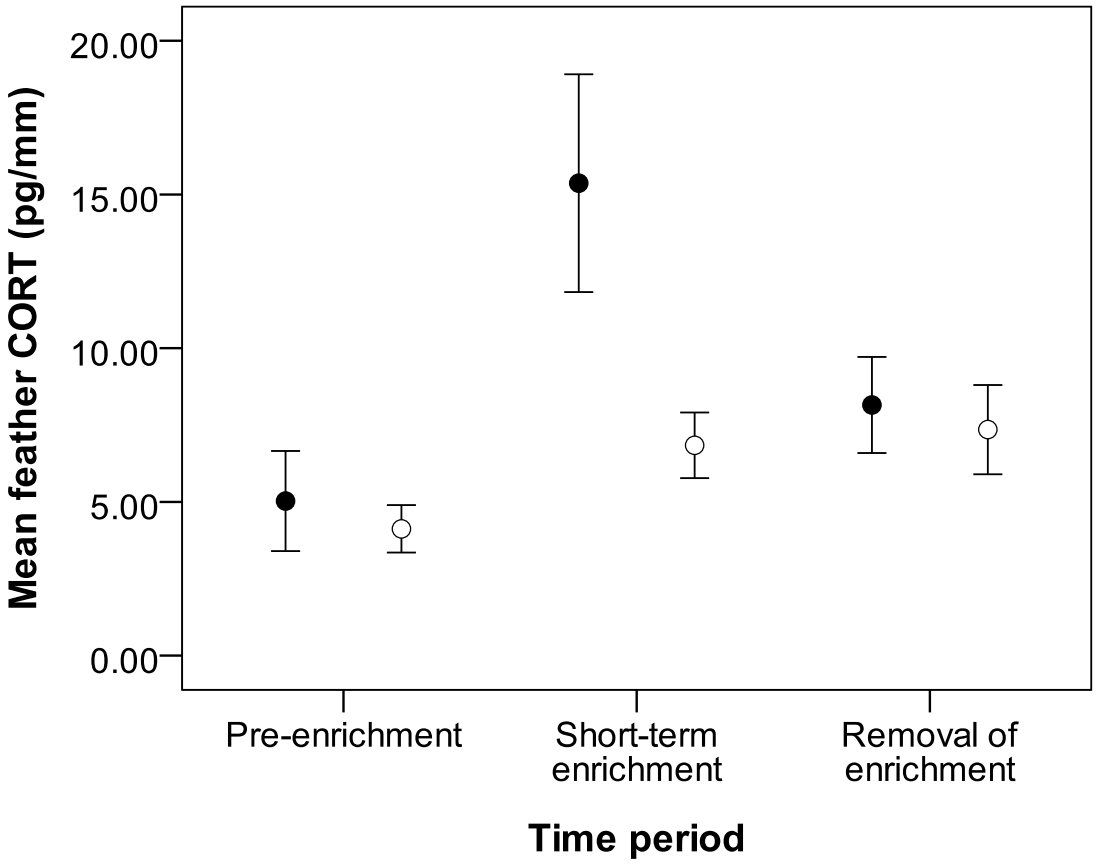


Figure 4.2. Mean (± SE) nutcracker feather CORT values (pg/mm) from experiment 1. Filled circles: experimental birds; open circles: non-enriched controls. See text for explanation of time periods.

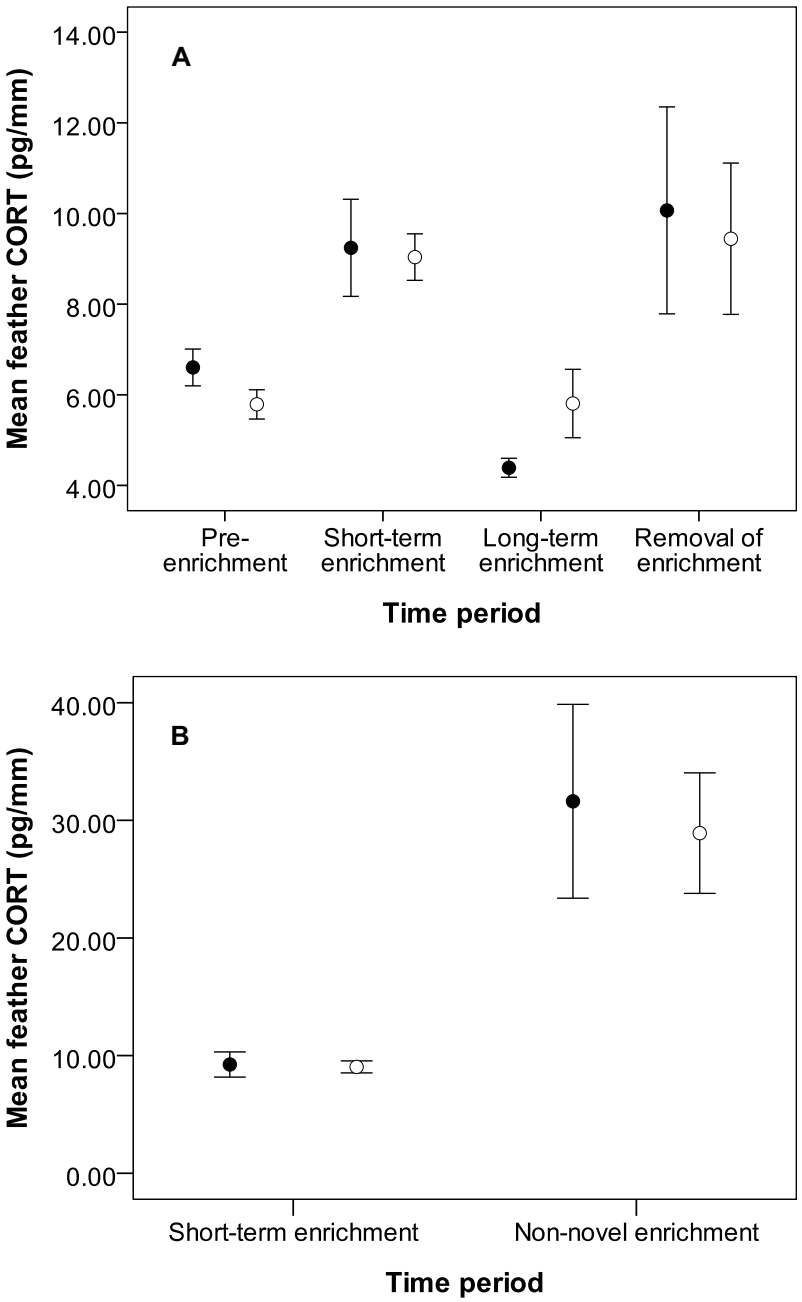


Figure 4.3. Mean (± SE) nutcracker feather CORT values (pg/mm) from experiment 2. Filled circles: experimental birds; open circles: non-enriched controls. (A) shows the first four time periods of the experiment; (B) shows the fifth time period (non-novel enrichment) and includes short-term (novel) enrichment for comparison. Note different scales on y-axes. See text for explanation of time periods.

but not in controls ($t_{1,93}$=-1.86, p=0.07). CORT levels increased significantly following non-novel enrichment for both control and experimental birds and were significantly higher than both pre-enrichment ($t_{1,93}$=-5.15, p<0.0001) and enrichment levels ($t_{1,93}$=-4.49, p<0.0001).

4.3.3. Behaviour

There was a significant interaction between the effects of treatment and time period on LTF ($F_{2,52}$=4.30; p<0.02). During short-term enrichment, LTF was significantly greater in experimental birds than in controls ($t_{1,52}$=-2.58; p=0.01; Fig. 4.4), but this effect disappeared during long-term enrichment when LTF in experimental birds was reduced ($t_{1,52}$=3.89; p=0.0003) to levels seen in controls. CORT was not significantly related to LTF in either group ($F_{1,52}$=1.33; p=0.25).

There was no significant interaction between treatment and time period ($F_{1,73}$=0.11; p=0.96) in our model of AL, so we interpreted the main effects directly. AL did not differ between time periods ($F_{1,73}$= 0.88; p=0.45; Fig. 4.5) or between treatments ($F_{1,73}$= 0.72; p=0.40), and was not significantly related to CORT ($F_{1,73}$= 0.50; p=0.48).

Our model of EXP revealed that, overall, experimental birds were more likely to show pecking behaviour than control birds (Wald chi-square=5.5; p<0.01; Fig. 4.6) and that pecking was significantly more likely during the short-term enrichment than in other periods (Wald chi-square=13.33; p<0.001). EXP was not related to CORT (Wald chi-square=0.26; p=0.61) for either group.

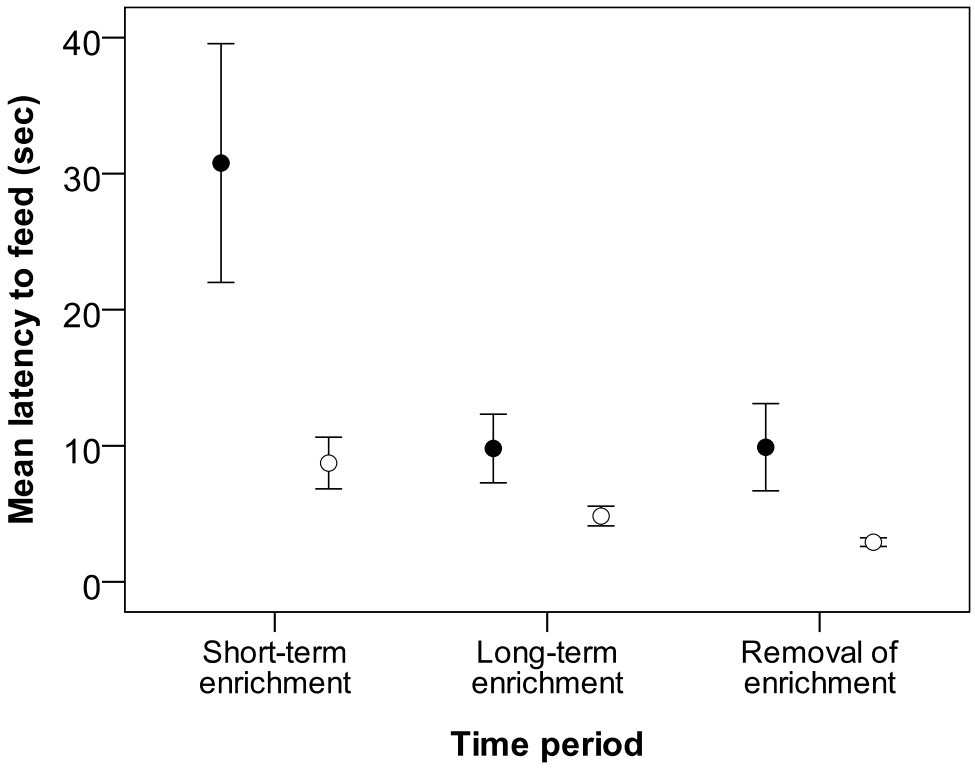


Figure 4.4. Mean (± SE) latency (sec) to feed (LTF) of nutcrackers during experiment 2. Filled circles: experimental birds; open circles: non-enriched controls. See text for explanation of time periods.

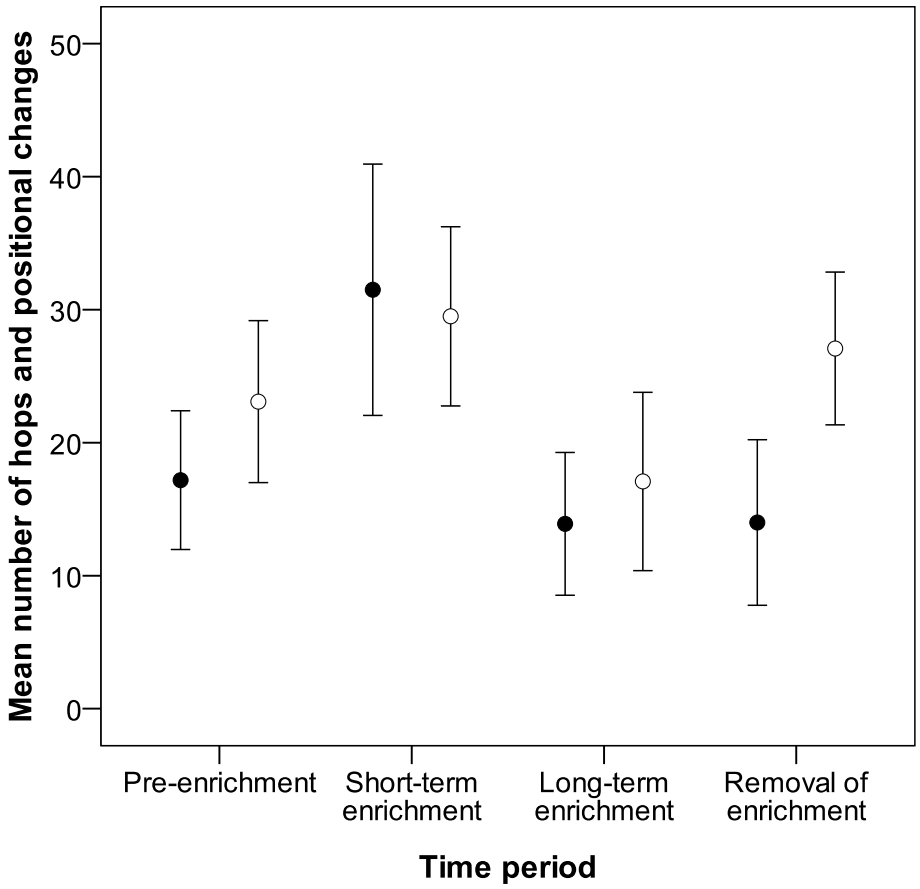


Figure 4.5. Mean (± SE) counts of nutcracker hops and positional changes (activity level, AL) during experiment 2. Filled circles: experimental birds; open circles: non-enriched controls. See text for explanation of time periods.

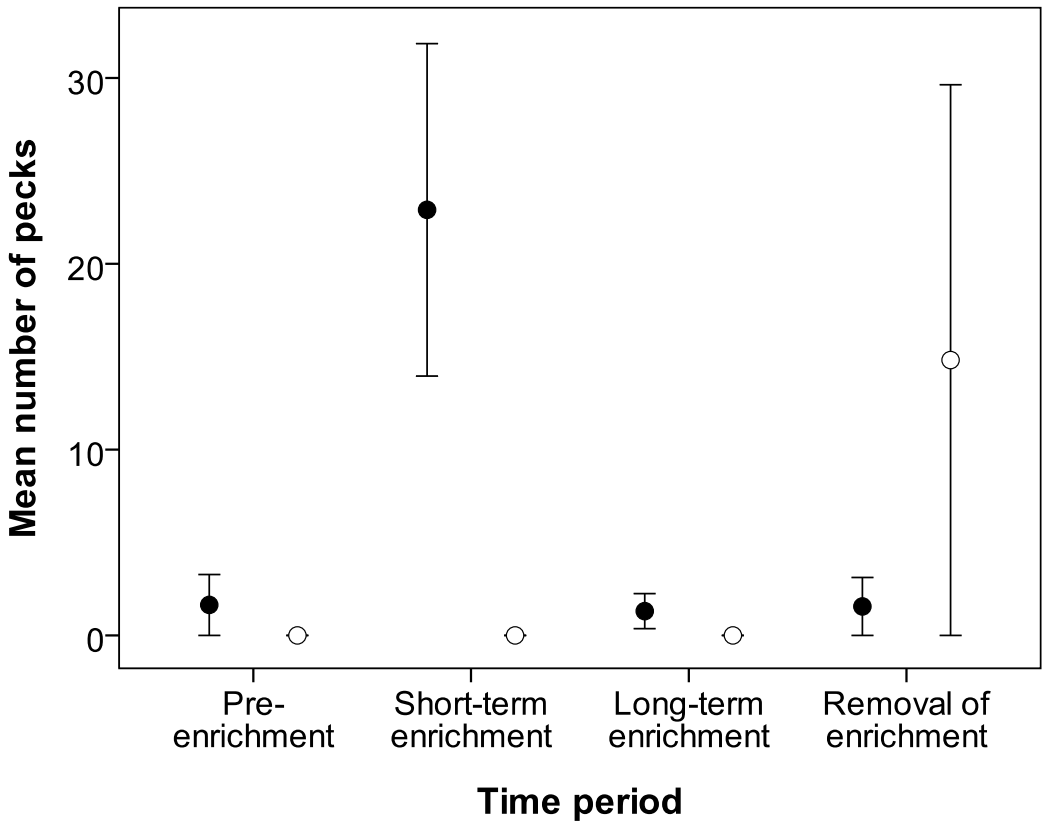


Figure 4.6. Mean (± SE) counts of nutcracker pecks (exploration, EXP) during experiment 2. Filled circles: experimental birds; open circles: non-enriched controls. See text for explanation of time periods.

4.4. Discussion

Did enrichment attenuate stress physiology in captive nutcrackers? The integrated measure of CORT increased significantly from pre-enrichment levels following short-term exposure to enrichment objects in two separate experiments, indicating that stress physiology was likely enhanced, not reduced, following the manipulation. However, individuals exposed to long-term enrichment (i.e., the final 25 days of a 92-d enrichment period) expressed CORT levels that were significantly lower than pre-enrichment values, suggesting a physiological benefit. These results led us to hypothesize that nutcrackers perceived initial enrichment as a stressful change of environment, and we predicted that the change of environment associated with removal of enrichment would likewise produce elevated CORT levels. Contrary to our predictions, CORT levels in experiment 1 decreased following removal of enrichment and returned to pre-enrichment levels. However, in experiment 2, CORT levels increased significantly to levels seen following short-term enrichment, in accordance with our predictions.

How do we explain these seemingly opposite responses to removal of enrichment in our two experiments? The main difference between experiments 1 and 2 was length of exposure to enrichment objects prior to removal. The longer exposure likely allowed birds in experiment 2 to habituate to the objects such that the objects became part of their normal environment, similar to acclimation seen in previous studies ([240,241] and see [89]). The fact that CORT levels during long-term enrichment (the final 25 days of a 92-d enrichment) were not significantly higher than those prior to enrichment provide evidence that birds had indeed adjusted to the presence of objects. Thus, removal of the objects in

experiment 2 constituted a change of environment to which birds responded by elevating CORT. This was not the case in experiment 1 where the shorter 10-day exposure period did not allow for the same length of adjustment. Objects were therefore not recognized as part of the environment and instead were perceived as a stressor. Thus, the removal of the objects was seen as a removal of a stressor, to which birds responded by decreasing CORT secretion, albeit non-significantly, to pre-enrichment levels.

Were the birds physiologically stressed by short-term enrichment? Although none of the birds showed any sign of illness or discomfort during either of our experiments, two birds died during, and one shortly after, experiment 2. Interestingly, all three fatalities were in the experimental group, and those individuals had three of the four highest CORT values for the short-term enrichment period. The provisioning of enrichment must be done carefully and in the context of the species and environment. It is possible that the installation of four objects inside the cage was perceived as an over-enrichment by nutcrackers, and the birds reacted with sustained CORT responses. Sustained elevated CORT secretion can suppress immune response [13,62], so elevated CORT levels may have been a contributing factor in the deaths. However, CORT values during short-term enrichment, when responses would be expected to be most robust, were not the highest seen in the experiment, so we doubt that birds were over-enriched. Thus, we cannot conclude that enrichment *per se* caused stress sufficient enough to result in these deaths.

Behaviourally, only LTF and EXP behaviour were influenced by the addition of enrichment objects, but these responses were not seen two months later, nor were they seen upon removal of the objects. By contrast, feather CORT increased significantly in periods following both the addition and removal of enrichment objects. Importantly, a lack

of relationship between feather CORT levels and concurrently measured behaviours support the idea that stress-related behaviour and GC physiology may act independently of each other within an individual, and highlights a physiological response that was not detectable through the behaviours we measured. Despite evoking a physiological response to enrichment, is possible that the objects were not engaging enough to elicit anything other than transient behavioural responses. This is in contrast to previous studies that found behavioural, but not physiological, responses to enrichment [203,204,217,218,221]. These results suggest that both context and type of enrichment are important determinants of responses to enrichment.

Control birds showed similar feather CORT levels to experimental birds in all but one period of experiment 2, despite not being able to see enrichment objects. Although we made every effort to ensure that control birds were treated identically to experimental birds other than not receiving enrichment objects, we cannot rule out the possibility that some factor common to both groups influenced our results. Controls were only visually, and not aurally, isolated from their experimental counterparts. Nutcrackers are semi-social corvids that possess complex vocal communication systems [235]. Nutcrackers in both treatment groups were noisy throughout the experiment, so it is possible that vocalizations from experimental birds were changed or interrupted by the presence of enrichment objects, and the perception of these altered vocalizations promoted a release of CORT by control birds. Public information can affect CORT levels in birds [242], so it is possible that vocalizations work in a similar way, alerting individuals. This intriguing possibility requires further research.

Our results challenge the notion that all types of enrichment are immediately beneficial for captive animals and indicate that initial reactions to enrichment need not be positive or, in the case of some behaviours, may even be absent. Furthermore, a lack of relationship between feather CORT and the behaviours we measured suggests that relying solely upon behavioural measures of stress to assess captive animal well-being can be misleading, an assertion supported by other studies [203,204,217,218,221]. We are not suggesting that enrichment is harmful to captive animals. In fact, our results indicate that long-term enrichment generally reduced activity of the HPA axis and therefore may provide some physiological relief for captive animals experiencing otherwise stressful conditions. This result was not seen in control birds, so the effect was likely caused by exposure to enrichment objects. Furthermore, the fearfulness we observed in response to short-term enrichment was not detected following long-term enrichment, suggesting that this effect was temporary.

Likewise, although we did not detect a behavioural response to removal of enrichment objects, we are not suggesting that impoverishment has no behavioural consequences. On the contrary, numerous studies have documented deleterious behavioural effects of impoverished environments (e.g., [217,218]). However, in our study we were only concerned with behavioural responses occurring very soon after experimental manipulations; as we did not detect any change in the behaviours we measured following removal of enrichment objects, we conclude that nutcrackers did not respond behaviourally to removal of enrichment.

Previous work has suggested that novelty is an important property of enrichment to which animals respond [197,200,238], but results have been mixed [243]. Our

experimental design allowed us to assess the effect of novelty on nutcracker GC secretion because birds in experiment 2 were re-enriched with objects to which they had previously been exposed. Had novelty been a factor influencing GC responses in the short-term enrichment period in experiment 2, feather CORT values during the non-novel enrichment period (i.e., second exposure to the objects) should have been lower than or equal to levels following initial exposure. To the contrary, we found feather CORT values to be significantly higher when birds were no longer naïve to the enrichment objects. This indicates that nutcrackers mounted a stronger physiological response the second time they were exposed to the same objects, a result previously seen only in mammals [243]. The non-novel enrichment period in our study was only 10 days, compared to 25 days for the short-term enrichment period, so it is plausible that the shorter growth period resulted in a higher average CORT. However, the feather sections in experiment 1 were also grown over 10 days, yet their CORT values were half that of the non-novel enrichment values in experiment 2 and comparable to the values from longer feather sections grown during experiment 2. Thus, it appears that the length of feather growth period is not responsible for the high CORT values seen during the non-novel enrichment period. As an alternative explanation, the experimental birds may have developed a negative association between the enrichment objects and the activities of our experiment. Although all nutcrackers in our experiment had been handled daily for several years prior to our experiment, the extra handling required for measuring feather lengths and collecting feathers may have been perceived as stressors by the birds. The enrichment objects may thus have acted as a cue and upon seeing the enrichment objects a second time birds responded more strongly because they had a negative prior experience.

4.4.1. Conclusions

Enrichment can undoubtedly alter both physiological and behavioural functioning in animals [210,213,216,223,244,245]. By demonstrating that an integrated measure of GC physiology varies significantly with changes to enrichment in the absence of agonistic interactions, our study sheds light on potential mechanisms driving those physiological and behavioural effects. Our work adds an avian perspective to studies addressing GC responses to novelty, and suggests that when a non-novel stimulus acts as a cue, acclimation may be overridden by negative past experience. Importantly, our findings suggest that how animals perceive enrichment and its removal depends on the duration of exposure: shorter-term enrichment may be experienced as a stressor, but longer-term enrichment allows for acclimation and therefore subsequent removal of enrichment constitutes a change to the environment. Future research should work to identify the factors that affect the rate at which individuals transition between these two psycho-physiological states. Studying such factors in captive and free-living animals will improve our understanding of how and why animals adapt to environmental change.

Chapter 5. Does post-breeding corticosterone reflect the expense of reproduction, individual quality, or both?[4]

5.1. Introduction

Reproduction is energetically demanding and parents must continually invest resources to ensure the survival of their offspring. Egg formation, incubation, and provisioning and defense of young require substantial energy, particularly for females of many species [246-248]. During reproduction parents must additionally maintain their own condition while also coping with an unpredictable environment. How individuals resolve energetic trade-offs during breeding is important because fitness can be reduced if energy is diverted to functions other than reproduction [19,249]. By studying physiological mechanisms that manage resource allocation within individuals, we can gain insight into how the energetic demands of reproduction contribute to variation in life histories [30].

Glucocorticoid hormones are involved in energy regulation, responses to stressors, and life history transitions [11,13-15] and therefore play an important role in adjusting parental physiology to the demands of rearing young [250]. Baseline levels of corticosterone (CORT), the primary avian glucocorticoid, increase when greater demands are put on females via brood size manipulations [70,251] and are highest in the most productive individuals of both sexes [252]. Additionally, cumulative female productivity within a breeding season is positively related to stress-induced levels of CORT measured post-breeding [72]. Increased levels of CORT are frequently associated with detrimental

[4] I gratefully recognize the contributions of G. Treen, R.G. Clark, and G.R. Bortolotti to this work.

consequences, including reduced reproductive behaviours [67]. However, the "CORT-adaptation" and "CORT-fitness" hypotheses [70,251] posit that if increased CORT facilitates increased parental behaviors, it should also result in greater reproductive investment and ultimately higher fitness.

We argue that CORT need not increase parental behaviour for the CORT-adaptation hypothesis to be supported. In fact, an attempt to establish this causal link using exogenous CORT was not successful [253]. Instead, if increased CORT were a consequence of energetic exertion, then more productive individuals would be predicted to have higher CORT. In this sense, CORT secreted during and after breeding may reflect, respectively, the short- and long-term energetic "expense" of raising offspring. In species such as tree swallows (*Tachycineta bicolor*) that moult once annually following breeding, CORT from feathers may quantify an individual's cumulative expense of raising young because it integrates both baseline and stress-induced CORT secretion into a biologically-relevant measure of total CORT secretion [105-107]. However, the ability to invest more in reproduction may depend on condition [70], suggesting that the relationship between feather CORT and reproduction may depend on quality.

Here, we explore the relationships between reproduction, individual quality, and CORT using feathers collected from adult tree swallows that bred consecutively in our study area in 2008 and 2009. We test predictions of two hypotheses. First, if feather CORT reflects the cumulative energetic expense of reproduction, as has been shown previously using plasma [72], then reproductive effort (clutch size and number of young fledged) in 2008 should be positively correlated with post-breeding CORT. Second, we hypothesize that quality should correlate positively with CORT because only better quality birds should

be able to tolerate the increased levels CORT. We therefore predict that feather CORT should covary with behaviors related to quality. Timing of reproduction and choice of nest site are important determinants of fitness in birds [254,255]. This is particularly true for secondary cavity-nesting species such as tree swallows because limited availability of good quality nest cavities (or artificial nest boxes) can result in strong competition [256]. Earlier breeding individuals can compete for better quality nest sites because they arrive earlier and tend to be of better quality [254,257,258]. Therefore, we predicted that 2008 clutch initiation date would be negatively related to feather CORT. The two types of nest boxes in our study area (plywood and aspen) differ in microclimate (Chapter 2) and cavity nesting species likely select nest sites based on stability of cavity microclimate [259]. Therefore, we predicted that individuals breeding in aspen boxes (more stable microclimate) would have significantly higher feather CORT. We additionally determined if 2008 CORT levels predicted the type of box used in 2009.

5.2. Materials and methods

5.2.1. Field methods

Work was conducted at St. Denis National Wildlife Area, SK, Canada, from May-August in 2008 and 2009 as part of an ongoing swallow population monitoring project. In both years, we erected 25 plywood nest boxes and 25 boxes constructed from aspen (*Populus tremuloides*) logs. 2008 was the first year that birds were offered aspen boxes. All

boxes were located in similar habitat and we alternated box type to avoid clustering either box type. Neither internal box chamber dimensions nor size of entrance hole differed between box types, but wall thickness was 1 cm in plywood boxes and ~4 cm in aspen boxes, resulting in a significantly more stable microclimate in the latter as measured by data loggers (Chapter 2).

Throughout the 2009 breeding season we captured, banded, and collected two flank feathers from 29 adults (15 m, 14 f) nesting in plywood boxes and 32 adults (14 m, 18, f) nesting in aspen nest boxes. Of these, 25 (13 m, 12 f) had bred in the study area in 2008, so we had data on type of nest box used, clutch initiation date (CID), clutch size (CS), number of young fledged (NF), wing length, and mass for those individuals in both years. Additionally, we calculated a body condition index (BCI) according to Green (2001) based on measurements of all birds in our population (> 400). Tree swallows molt only once annually, so the feathers we collected in 2009 were grown post-breeding (i.e., Aug – Sept) in 2008. However, CORT in feathers is stable over time [106]. All collected feathers were stored in plain paper envelopes until extraction for CORT analysis.

5.2.2. Corticosterone analysis

Extraction of CORT from feathers followed [105] and has been replicated with tree swallows [132] and other species [65,107]. We measured the length of each flattened feather against a metal ruler. We removed and discarded the calamus and remeasured the feather length. Each feather was placed in a separate glass vial. We then cut feather samples into very small pieces, added 10 mL of methanol (HPLC grade, Fisher Scientific,

Fairlawn, New Jersey, USA) to each sample, placed the samples in a sonicating water bath at room temperature for 30 min, and incubated them at 50 °C overnight in a water bath. Using a plug of synthetic polyester fibre inserted into a filtration funnel, we separated the methanol from feather bits using vacuum filtration. Methanol extracts were then placed in a 50 °C water bath and evaporated under a fume hood, reconstituted in a small volume of phosphate buffered saline (0.05M, pH 7.6), and frozen at –20 °C until analyzed by radioimmunoassay (RIA). We assessed the efficiency of the methanol recovery using three additional feather samples each spiked with a small amount (approximately 5000 CPM) of ^{3}H-corticosterone in the extraction. Samples used for recovery efficiency were sonicated, incubated, and filtered as above. For additional information about extraction validation, see online Supplementary Appendix S1 in (Bortolotti et al. 2008). Samples were extracted in one batch, with >97% of the radioactivity being recoverable from reconstituted samples.

Reconstituted sample residues were analyzed by radioimmunoassay (in duplicate) by incubating 200 µL of antiserum with 100 µL of extracted samples (or standards) and 100 µL (approximately 5000 CPM) of ^{3}H-labeled corticosterone for at least 16 hrs at room temperature. We used a dextran-coated charcoal stripping technique to separate bound and free hormone. We purchased antiserum and purified CORT for standards from Sigma Chemicals, ^{3}H CORT from Amersham Bioscience. We measured samples in one assay with an intra-assay coefficient of variation of 8.4% and a detection limit of 4.88 pg CORT/assay tube, but all data values were above this limit and we had no undetectable samples. CORT deposition in feathers is time-dependent [108], so data are expressed as pg CORT per mm of feather, which gives a valid estimate of CORT per unit time of feather growth [105,106]. Assays were performed at the University of Saskatchewan, Canada.

5.2.3. Statistical analyses

To improve normality, we log transformed feather CORT data. A single CORT value was greater than 1.5 interquartile ranges lower than the first quartile and was removed from analyses as a statistical outlier. Additionally, one individual in our sample raised only two young, which is out of the norm for this population (R. G. Clark, pers. obs). Importantly, this bird likely had significantly reduced parental effort relative to other individuals in our study [260]. We therefore excluded this individual from analyses.

Our general statistical approach followed previous studies [72,252]. We fit generalized linear models (GLM) to the data using SAS v. 9.1 (SAS Institute, Cary, NC, USA). To account for the clustering of our data by nest, we included nest identity as a random statement using PROC GLIMMIX, but these models would not converge. We therefore considered the sexes separately in GLMs using PROC GENMOD.

5.2.3.1. Box type and individual variation in quality

We first tested for possible differences between birds that could be attributed to box type (plywood or aspen) in 2008. We considered CORT, BCI, log-transformed CID, and wing length (a predictor of timing of breeding in tree swallows; [261]) separately as dependent variables in GLMs. Because attempts at normalizing NF failed and parameter estimates for model terms were unreasonably high using GLMs, we compared 2008 CS and NF between box types using separate nonparametric independent-samples Kruskal-Wallis tests. CORT, BCI, CID, wing length, CS, and NF did not differ between box types used in 2008 for either

sex (see Results); box type was therefore not considered in subsequent models for 2008 reproductive parameters. We determined if CS and NF differed by box type in 2009 using GLMs.

Next, we assessed individual variation in quality. We determined if earlier breeders had larger clutches by fitting GLMs with log-transformed CID as the dependent variable and CS as a fixed factor. This model was repeated for NF to determine if earlier breeders were more productive. We also determined if wing length was a significant predictor of CID. We then determined if reproductive parameters (CID, CS, NF) were consistent within individuals between breeding seasons. For CID, we used GLMs and assigned log-transformed CID in 2008 as the dependent variable and included 2009 CID as a fixed factor. CID was standardized relative to the earliest date in each sample for each year, which was within the norm for our population (R.G. Clark, pers. obs.). Attempts at normalizing CS and NF data were not successful, so we used two-tailed Spearman correlations.

5.2.3.2. Relationships between reproduction and CORT

To characterize the relationships between reproductive parameters (CID, CS, and NF) measured in 2008 and CORT, we fit GLMs to the CORT data. We created three models, one each for CID, CS, and NF as main effects. We chose to analyze reproductive parameters separately to avoid possible variance inflation errors arising from including collinear predictor variables [262] and also to maximize power of statistical tests because sample sizes were small.

To test for a relationship between CORT and NF while controlling for quality, we ran three GLMs. Each used CORT as the dependent variable and included NF and one of the following covariates: CID (with reservations about including collinear predictor variables), wing length, and BCI.

5.2.3.3. CORT and subsequent choice of nest box

To determine how CORT related to nest box use in 2009, we fit a GLM with CORT as the dependent variable and 2009 nest box type as a main effect. We further explored this relationship with a GLM that used CORT as the dependent variable, whether or not an individual switched nest box type between years (yes or no). We then expanded our sample to consider nest box use of all 61 birds for which we had feather CORT data in 2009 (i.e., 35 birds that bred in the study area in 2009 only plus the 25 birds that bred in the study area both years). To improve normality, we used log-transformed CORT data. One data point was 1.5 interquartile ranges lower than the first quartile and another was 1.5 interquartile ranges higher than the third quartile, so both were omitted from analysis as statistical outliers. With this bigger sample size we were able to fit a generalized linear mixed model using PROC GLIMMIX with nest identity as a random factor to account for potential clustering associated with individual nest boxes. In this model we included log-transformed CORT as the dependent variable, 2009 box type as a main effect, sex as covariate, and a sex × main effect interaction term.

All GLMs were fit using a normal distribution and an identity link function. We used a backward stepwise approach of eliminating non-significant model terms from full models to arrive at final models that included at least the main effect.

5.3. Results

5.3.1. Box type and individual variation in quality

Feather CORT, BCI, CID, and wing length did not differ between plywood and aspen boxes for either sex in 2008 (Table 5.1). Models combining the sexes revealed the same results (CORT: $\chi^2_{1,21} = 0.20$, $p = 0.65$; BCI: $\chi^2_{1,20} = 2.28$, $p = 0.13$; CID: $\chi^2_{1,21} = 3.38$, $p = 0.07$; wing length: $\chi^2_{1,20} = 1.12$, $p = 0.29$). Similarly, neither CS (Kruskal-Wallis tests: males, $p = 0.93$; females, $p = 0.09$) nor NF (Kruskal-Wallis tests: males, $p = 0.66$; females, $p = 0.07$) differed significantly between box types for either sex in 2008. We therefore conclude that the different box types did not attract significantly different quality birds in 2008. Similarly, in 2009, neither CS (males: $\chi^2_{1,10} = 0.24$, $p = 0.63$; females: $\chi^2_{1,9} = 0.09$, $p = 0.77$) nor NF (males: $\chi^2_{1,10} = 0.08$, $p = 0.77$; females: $\chi^2_{1,9} = 0.32$, $p = 0.57$) differed significantly between box types.

Earlier breeding females, but not males, had significantly larger clutches (Table 5.2). Similarly, earlier breeding females, but not males, fledged significantly more young (Table 5.2). Wing length was not significantly related to CID for females ($\chi^2_{1,9} = 0.00$, $p = 0.95$), but

Table 5.1. Model results for the effect of box type (plywood or aspen) on male and female tree swallow corticosterone (CORT), body condition index (BCI), clutch initiation date (CID), and wing length. Coefficients are relative to aspen boxes.

	coefficient	**s.e.**	**χ^2 (d.f.)**	**p-value**
males				
CORT	-0.047	0.054	0.72 (1,10)	0.40
BCI	-0.014	0.011	0.56 (1,10)	0.21
CID	-0.015	0.009	2.32 (1,10)	0.13
wing length	2.314	2.527	0.81 (1,10)	0.37
females				
CORT	0.014	0.046	0.09 (1,9)	0.76
BCI	-0.013	0.014	0.80 (1,9)	0.37
CID	-0.023	0.020	1.26 (1,9)	0.26
wing length	0.500	0.736	0.45 (1,9)	0.50

Table 5.2. Model results for the relationships between clutch initiation date and clutch size (CS) and number of young fledged (NF) for male and female tree swallows. Significant effects are in bold.

	coefficient	**s.e.**	**χ^2 (d.f.)**	**p-value**
males				
CS	0.002	0.009	0.08 (1,10)	0.78
NF	0.011	0.006	2.89 (1,10)	0.09
females				
CS	-0.029	0.007	16.62 (1,9)	**< 0.0001**
NF	-0.022	0.008	6.22 (1,9)	**0.01**

earlier breeding males had significantly longer wings ($\chi^2_{1,10}$ = 16.33, p < 0.0001). There was no within-individual relationship for CID between 2008 and 2009 for either sex (males: $\chi^2_{1,10}$ = 1.69, p = 0.19; females: $\chi^2_{1,9}$ = 0.16, p = 0.69; sexes combined: $\chi^2_{1,21}$ = 2.52, p = 0.11). However, females with bigger clutches in 2008 also had bigger clutches in 2009 (Spearman's rho = 0.80, p = 0.003), whereas this relationship was not significant for males (Spearman's rho = -0.17, p = 0.60). Parents that were more productive in 2008 were also more productive in 2009 (Spearman's rho = 0.58, p = 0.004), but this effect was significant for males only (males: Spearman's rho = 0.70, p = 0.01; females: Spearman's rho = 0.36, p = 0.28) when the sexes were considered separately.

5.3.2. Relationships between reproduction and CORT

CID was not related to CORT for males (Table 5.3; Fig. 5.1a). By contrast, earlier breeding females had significantly higher CORT (Table 5.3; Fig. 5.1a). Although three data points (1 male, 2 female) with CIDs later than day 160 appear to influence this relationship, removal of these data did not change the model direction or significance (males: $\chi^2_{1,10}$ = 1.74, p = 0.19; females: $\chi^2_{1,7}$ = 3.93, p < 0.05; Fig. 5.1b). Females, but not males, with larger clutches had significantly higher CORT (Table 5.3; Fig. 5.1c). Female, but not male, CORT was significantly positively related to NF (Table 5.3; Fig. 5.1d). After controlling for CID, CORT was not related to NF for either sex (Table 5.4). Controlling for wing length or BCI did not change the relationship between CORT and NF for either sex (Table 5.4). If anything, controlling for wing length appeared to strengthen the relationship for females.

Table 5.3. Model results for the influence of clutch initiation date (CID), clutch size (CS), and number of young fledged (NF) on feather CORT for male and female tree swallows. Significant effects are in bold.

	coefficient	**s.e.**	**χ^2 (d.f.)**	**p-value**
males				
CID	0.008	0.004	3.13 (1,10)	0.08
CS	0.025	0.046	0.30 (1,10)	0.58
NF	0.061	0.034	2.91 (1,10)	0.09
females				
CID	-0.005	0.001	11.76 (1,9)	**0.0006**
CS	0.040	0.018	4.22 (1,9)	**0.04**
NF	0.044	0.018	4.84 (1,9)	**0.03**

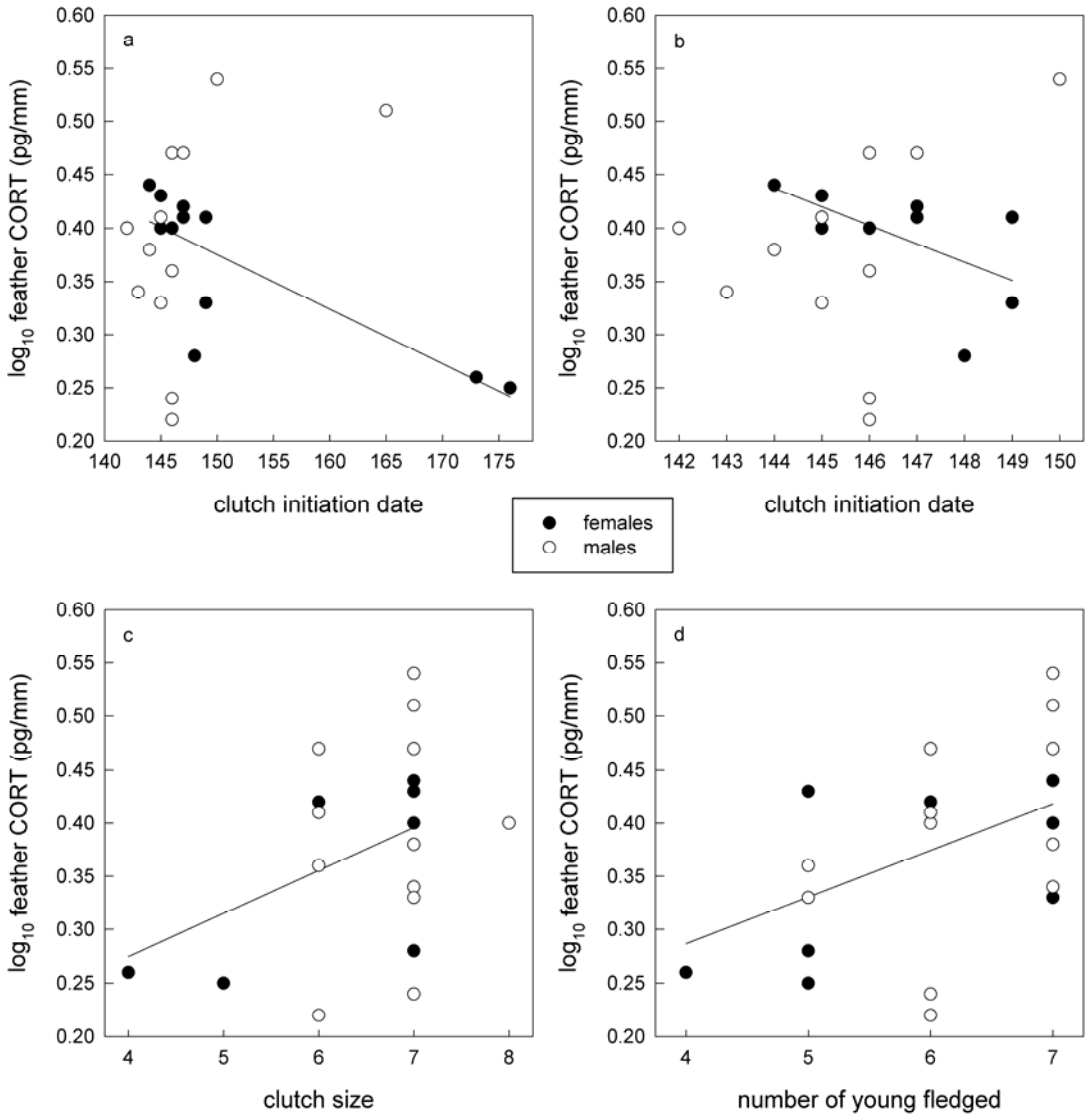


Figure 5.1. Relationships between 2008 reproductive parameters and feather CORT of adult tree swallows. Note that (b) shows same data as (a) but only for individuals with clutch initiation date < 160. See text for explanation. Lines shown are for female data only and are all significant at $p < 0.05$.

Table 5.4. Model results for the relationships between number of young fledged (NF) and CORT after controlling for the effect of clutch initiation date (CID), wing length, and body condition index (BCI). Coefficient, s.e., and statistics presented are for NF with covariate in the model.

	controlling for	**coefficient**	**s.e.**	**χ^2 (d.f.)**	**p-value**
males	CID	0.045	0.033	1.73 (1,9)	0.19
	wing length	0.043	0.035	1.42 (1,9)	0.23
	BCI	0.048	0.039	1.48 (1,9)	0.22
females	CID	0.008	0.017	0.23 (1,8)	0. 63
	wing length	0.065	0.012	13.76 (1,8)	**0.0002**
	BCI	0.044	0.018	4.87 (1,8)	**0.03**

5.3.3. CORT and subsequent choice of nest box

Females breeding in plywood boxes in 2009 had significantly higher CORT than females breeding in aspen boxes ($\chi^2_{1,9} = 12.70$, $p = 0.0004$; Fig. 5.2a). Females that switched box types between years had significantly lower CORT than females that used the same box type ($\chi^2_{1,9} = 9.35$, $p = 0.002$; Fig. 5.2b). There was no significant difference between males in the two box types in 2009 ($\chi^2_{1,10} = 2.02$, $p = 0.15$) and males that switched box types between years were statistically indistinguishable from those that used the same box type ($\chi^2_{1,10} = 0.28$, $p = 0.60$). When we increased our sample to include all birds for which we had feather CORT data, we found a strong relationship between CORT and box type: individuals that bred in aspen boxes had significantly lower CORT than those that bred in plywood boxes (GLIMMIX: $F_{1,26} = 49.66$, $p < 0.0001$; Fig. 5.3). Neither sex nor the interaction term was significant in this model (both $p > 0.74$).

5.4. Discussion

The relationships between reproduction, individual quality, and CORT are complex. We used an integrated measure of GC physiology relevant to the post-breeding period to provide a different perspective than that of previous studies. Did feather CORT measure the expense of reproduction, individual quality, or both? Our results suggest that although feather CORT likely measured the expense of reproduction, that expense could not be separated from individual quality. We argue that reproductive effort may be matched to individual physiology based on quality. In this context, individuals trade off the benefit of

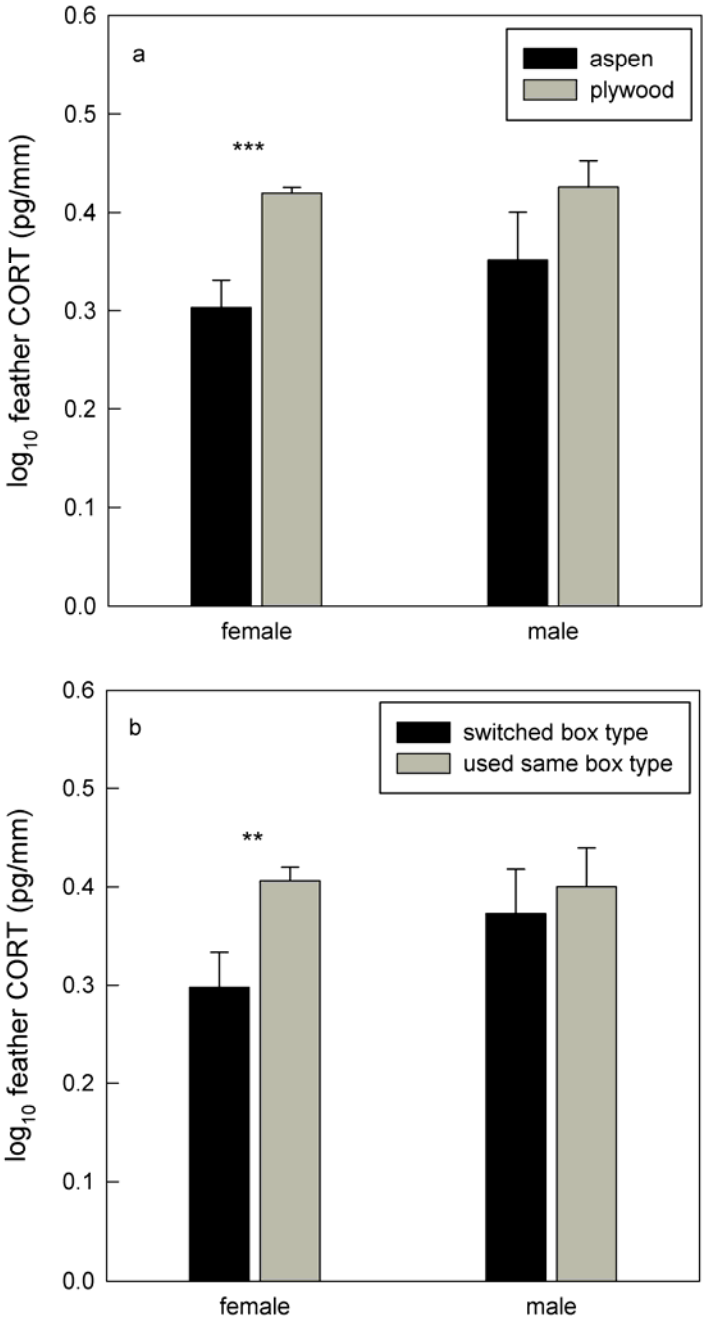


Figure 5.2. Average (± SE) feather CORT of 23 adult tree swallows that bred in the study area in 2008 and 2009 in relation to (a) box type used for breeding in 2009 and (b) whether or not an individual bred in the same or different box types in 2008 and 2009. *** $p < 0.001$, ** $p < 0.01$

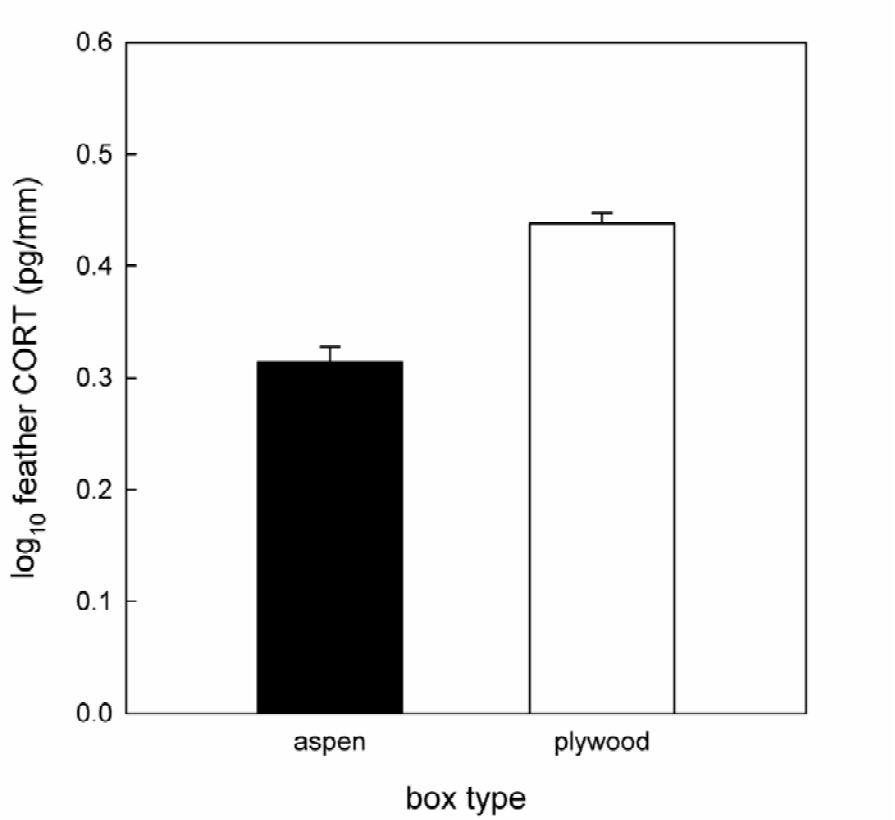


Figure 5.3. Average feather CORT (± SE) of 59 adult swallows in relation to box type used for breeding in 2009. Bars represent one standard error. Groups differed significantly at $p < 0.0001$.

raising additional young against the physiological cost entailed by elevated levels of CORT required to fuel increased provisioning and protection. This is in line with the prediction of the CORT-adaptation hypothesis that only better quality birds should have increased levels of CORT [251]. In fact, we suggest that tolerance to the detrimental effects of elevated CORT may be part of what makes a good-quality bird. Though untested, this idea may help put life-history trade-offs into a better physiological context [30].

Female, but not male, productivity was positively related to CORT, even after controlling for wing length and condition. Parents provisioning larger broods likely had to work harder [263] and would require more energy to do so [264]. We reason that CORT would facilitate the general increase in energy demand required to raise larger broods, and increased CORT post-breeding was a consequence of increased parental effort, rather than a direct cause. This may explain how CORT can be related to productivity [251,252], but not directly affect provisioning [253]. Our results are also in accordance with the temporal trend observed in the relationship between CORT and fitness [70]. Later in the breeding period, CORT and fitness are expected to positively covary [70]. Our results and those of others [72,105] extend that temporal trend to include post-breeding and provide support for the hypothesis that CORT from feathers grown post-breeding reflects the expense of reproduction.

However, individual quality clearly played a role in the CORT-reproduction relationship. We addressed behavioral measures of quality before (i.e., CID, nest box use in 2008) and after (i.e., nest box use in 2009) feather growth, and predicted these behaviors would be related to CORT. Individual productivity was consistent between years, and birds that bred earlier were more productive, suggesting that reproduction was related to

individual quality. Importantly, CID and CORT were strongly related and after controlling for CID we found no relationship between CORT and productivity. Earlier arriving birds have been shown to have higher CORT due to environmental conditions [265], but it is unlikely that such an explanation would apply to the post-breeding period. We interpret our results as supporting our hypothesis that CORT covaries with quality.

Contrary to our prediction, neither individual quality nor CORT differed between box types in 2008, the first year that aspen boxes were available in our study area. However, feather CORT apparently predicted the type of nest box in which an individual bred the subsequent year (2009) and this was related to quality. Individuals that bred in a different box type in 2009 (i.e., switched box types) had significantly lower feather CORT, bred later, and were less productive in 2008. Changing nest sites can be function of poor reproductive success [266], so perhaps this is not a surprising result. Regardless, when we expanded our sample to include all individuals breeding in 2009 for which we had feather CORT, we discovered strong segregation in the two box types based on CORT. Individuals of both sexes breeding in aspen boxes had significantly lower CORT than birds breeding in plywood boxes. We expected the opposite result because the microclimate of aspen boxes is more stable than that of plywood boxes (Chapter 2) and presumably more desirable. Interestingly, this is the same pattern of CORT previously detected for nestlings raised in these boxes in 2009 (Chapter 2). While this segregation may be related to quality, there were no differences in productivity between box types in 2009. It is therefore unlikely that we would detect differences in productivity because all birds in our sample survived the winter and we were sampling the "best" birds in our study area. Instead, perhaps CORT varied with a general type or personality of bird [267,268] that preferred one box type or

another. CORT responses have been shown to be lower in "proactive" birds that tend to explore more readily and show less fearfulness towards novelty (see [43] for a review). It is interesting to note that the birds in our study that switched box types (i.e., bred in the face of novelty) in 2009 had the lowest CORT. Perhaps decisions to switch nesting sites in a subsequent year are partly driven by personality.

Our result that CORT from feathers grown post-breeding is an indicator of reproductive effort provides some insight into the mechanism behind the CORT-adaptation hypothesis [70]. Our findings also indicate that CORT may be proxy for quality in species that molt once annually following breeding. When taken together, these results suggest that feather CORT may be a measure of individual state following breeding. Feather CORT may therefore be useful for quantifying carry-over effects into the non-breeding season [269]. Future research should study the consequences of post-breeding variation in feather CORT, and whether this can be linked to migration or arrival on wintering grounds.

Chapter 6. Can feathers help us better understand habitat-physiology relationships?[5]

6.1. Introduction

Physiology mediates the relationship between animals and their environment [30]. Physiological mechanisms that underpin responses to environmental change are particularly important to conservation because they can inform us about how animals respond to and function within changing habitat. Habitat loss, fragmentation, and degradation (hereafter "habitat change") is a main driver of species declines and extinctions [270,271], yet we know very little about how individuals perceive, cope with, and respond physiologically to habitat change and how this may contribute to population trends. Complex interactions among habitat structure, food availability, predation, and competition make it difficult to interpret the influences of habitat change on individual animals.

The need for an effective biomarker of habitat change has led to the use of measures of glucocorticoid hormones (GCs) in habitat studies [40,41,272,273]. Generally, exposure to stressors (e.g., severe weather, predation, parasite infestation) results in an increase in GCs aimed at helping the animal cope with adverse conditions [13,33,36,39,89], though GC levels can decrease in response to some challenges [35,134,274]. Importantly, the sensitivity of GCs to environmental change [13,89] and significant relationships between GCs and components of vertebrate health, reproduction, and fitness [84,85,252] make GCs an important physiological link among habitat change, population dynamics, and ultimately conservation [272].

[5] I gratefully recognize the contributions of M. Vögeli, D. Serrano, J.L. Tella, A. Delgado, and G.R. Bortolotti to this work.

Limited research with mixed results indicates that the relationship between habitat change and GCs is far from clear, and our understanding of how vertebrates respond physiologically to habitat change is therefore limited [273,275]. This may be due, in part, to how previous GC studies have measured habitat. Virtually all previous habitat-GC studies have used either (i) a dichotomous habitat classification (disturbed/not disturbed, fragmented/continuous; but see [41]), which sheds little light on the actual factors that influence individual levels of GCs, or (ii) habitat measures at scales which may not be the most appropriate scale for physiological studies [for a review see 273]. Furthermore, all previous studies of habitat-GC relationships have used blood or fecal measures of GCs, which only provide short-term measures over hours or days and can be logistically challenging in the field. Thus, conservation would benefit from a long-term integrative GC biomarker of habitat, and particularly one that is more easily used in the field.

We suggest that the novel approach of synchronising feather-based measures of GCs and stable isotopes (SIs) may clarify the relationships between habitat change and GCs. Feathers contain a record of both corticosterone (CORT, the primary avian GC) and SIs over the period of feather growth [105,108,276,277]. CORT in feathers ($CORT_f$) is positively correlated with blood levels of CORT [105,107] and integrates the magnitude and duration of GC responses, including both baseline and responses to stressors. Thus, $CORT_f$ provides a long-term measure of avian physiological responses to environmental variation. $CORT_f$ values are positively correlated with capture and restraint and small-scale environmental change [105,133], and can negatively vary with nutritional condition [134]. However, no previous study has addressed how $CORT_f$ relates to habitat change.

SIs complement $CORT_f$ by providing an individual measure of local environment through signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). When tissues, such as feathers, are produced they incorporate carbon and nitrogen from food sources. In terrestrial systems, $\delta^{13}C$ values can be used to differentiate photosynthetic pathways because C_3 plants have smaller (i.e., more negative) $\delta^{13}C$ values than C_4 plants [278]. Thus, $\delta^{13}C$ values in feathers can reliably reflect the food chain from which individuals were feeding [277,278]. $\delta^{15}N$ values in feathers can be enriched (i.e., greater) with each step in trophic level, in xeric habitats relative to more mesic ones, and with agricultural intensification [277-279].

Combining feather-based measures of CORT with SIs provides a particularly strong approach to conservation because it can supply information about environmental conditions and an integrated physiological response to those conditions over the same time period. Here, we test the utility of combining $CORT_f$ with SIs as a measure of individual responses to environmental conditions and habitat change in a fragmented metapopulation of Dupont's larks *Chersophilus duponti* (henceforth "lark"), an endangered songbird whose semi-arid steppe habitat is being lost to agriculture. We begin by investigating the influence of spatial scale on the distribution of $CORT_f$ in the metapopulation using a spatial autocorrelation analysis. We then test four predictions of the hypothesis that $CORT_f$ should be related to direct and indirect (i.e., SIs from feathers) measures of the local environment. Extensive research has shown that adverse environmental conditions generally result in an increase in GCs [for reviews see 13,89]. Thus, we predict that (i) larks surrounded by a relatively greater proportion of agriculture, and individuals living in relatively hotter and drier habitats, should have relatively higher $CORT_f$ values. Related to this, we expect (ii) values of both $\delta^{13}C$ and $\delta^{15}N$ to positively correlate with the extent of agriculture,

particularly in relatively arid local environments. Therefore, (iii) $CORT_f$ should be positively related to both $\delta^{13}C$ and $\delta^{15}N$. Habitat specialists such as larks tend to crowd in remnant habitat patches [280], and crowding can adversely affect the physiology of free-living birds [281,282]. Thus, we predict that (iv) larks from smaller, more isolated, and more densely populated habitat patches should have relatively higher $CORT_f$. We conclude by integrating all this information to contrast how GCs relate to habitat metrics at two spatial scales (local and landscape) and with varying environmental conditions.

6.2. Materials and methods

6.2.1. Study system and field methods

Fieldwork was carried out in the Ebro Valley (NE Spain, Fig. 6.1) between 2004 and 2006. The climate is semiarid with little seasonal precipitation and maximum temperatures regularly exceeding 40 °C [283,284]. Lark habitat is restricted to flat steppes with natural vegetation, and habitat change over the past few decades has led to a scattered distribution of predominantly small local populations in discrete habitat patches within a landscape matrix dominated by agriculture (see Fig. 6.1) [280]. Larks are almost exclusively insectivorous but feed on a broad spectrum of arthropod families [285,286]. Habitat change is negatively affecting lark population dynamics, and dispersal appears to be very limited [280,287-289].

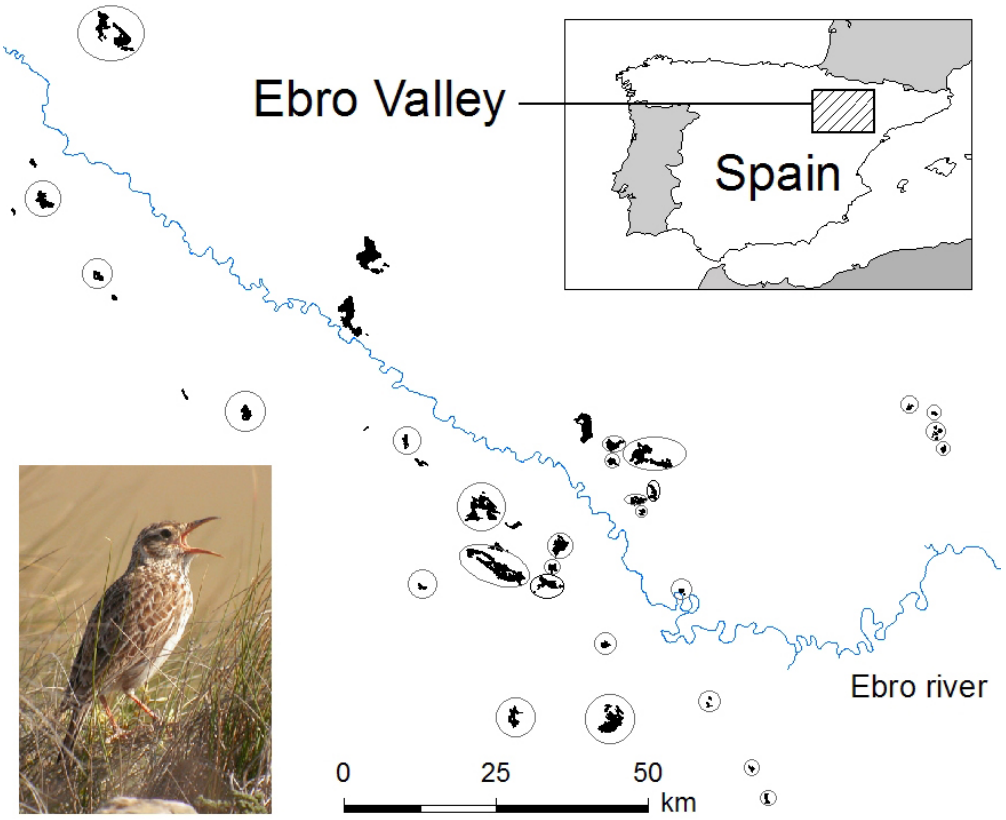


Figure 6.1. Map of the Ebro Valley study area in northeast Spain with inset photo of Dupont's lark. Remnant habitat patches occupied by larks are shown in black. Solid lines encircling patches identify the local populations sampled.

Larks were captured, ringed, and measured as described in [290]. Because we used playbacks of recorded vocalizations to capture larks, over 90% of captured individuals were male. Thus, we omitted juvenile and female larks from our analyses. During ringing, we collected a fully grown (dead) outer tail feather (6th rectrix) from each bird and additionally extracted a drop of blood for molecular sexing [290]. For both the 2004 and 2005 breeding seasons, we trapped some individuals in the fall after the post-breeding moult and others the following spring. Thus, the feathers collected from both seasons within one "feather-year" (i.e., fall 2004/spring 2005 and fall 2005/spring 2006) were grown during the same time period because larks moult only once per year in the fall [291]. The stability of $CORT_f$ is not affected by feather age [106].

To characterize habitat and spatial structure of the Ebro Valley metapopulation, we used variables described in Table 6.1. Lark habitat patch sizes were identified by the intersection of all lark locations obtained during field work in the Ebro Valley from 2004 to 2007 (n=2035; [280]) with highly detailed land-use maps [292]. Based on high-resolution aerial ortophotographs (1:5000, year: 2006) we adjusted these areas by discarding non-suitable steppe habitat for larks because of exceeding slopes (i.e. steeper than 5%, [293]). Steppe patches are easily recognizable at a landscape scale since they are island-like remnants in a landscape dominated by agriculture with evident boundaries to other land uses. Finally, all resulting habitat patches were digitalized to calculate their patch size in ArcGIS 9 [294]. Using CORINE land cover digital maps from the European Environmental Agency (year: 2000, map resolution = 100m), we calculated the percentage of land-use cover in the landscape matrix inside a buffer ring with 20km-radius (i.e., the maximum dispersal distance of larks detected in our study area [280]) from the patch edge.

Table 6.1. Variables characterizing the habitat and spatial structure of the Ebro Valley metapopulation of Dupont's larks. All variables were previously described by [280] unless noted otherwise

description	definition
vegetation community of habitat occupied by larks (veg6)	six phytosociological classes based on [295]
matrix land-use cover	percentages of land-use cover in the landscape matrix within 20 km (maximum dispersal distance of Dupont's larks) from each population edge
intensive agriculture (INTA) non-irrigated arable land (NIAL) natural steppe vegetation (NVEG)	these three main land-use categories described 83-97% of the total matrix cover
patch size	size (ha) of habitat patches
local population size	number of occupied territories within each local population
local population density	number of occupied territories per ha
NND	nearest neighbour distance, defined as the mean straight-line distance between the nearest singing neighbour [287]
isolation index	$I_i = - \Sigma \exp (- d_{ij}) N_j$, where d_{ij} is the Euclidean distance between populations i and j, and N_j is the number of occupied territories of population j [296]

The isolation (or inversely, connectivity) of each habitat patch was characterized as (i) the distance to the nearest occupied habitat patch and (ii) the isolation index $I_i = - \Sigma \exp(-d_{ij}) N_j$, where d_{ij} is Euclidean distance between patches *i* and *j*, and N_j is the number of occupied territories of patch *j* [296]. Lark population sizes were calculated as the number of occupied territories through territory mapping of males aided by its acoustic identification and observations of individually colour-ringed birds [289]. Lark breeding densities in each habitat patch were characterized as (i) the number of occupied territories divided by patch size, and (ii) the mean distance between the nearest singing male neighbours [280,287].

Data on the total measured precipitation during the lark moulting period (PDM) of July-September, and average maximum daily temperatures ($TMAX_{moult}$) during the same period, were obtained from regional administration websites [283,284]. Weather data was collected from nine meteorological stations across the study area, and we used weather data from the station nearest to each local population (mean distance: 10.5 km, range: 0 – 20 km).

6.2.2. Corticosterone analysis

$CORT_f$ assays followed methods in [105] and have been replicated with other species [132-134]. Each feather was measured from end to end (i.e., proximal calamus to distal tip of the vane) by flattening and straightening the entire feather length against a metal ruler. The calamus was then removed and discarded and the remaining sample feather length was remeasured as above. Each feather was then placed in a separate glass

vial and cut into pieces <5 mm^2 with scissors. We extracted CORT from feathers by adding 10 mL of methanol (HPLC grade; Fisher Scientific, Fairlawn, New Jersey, USA) to each vial and placing the samples in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight. Methanol was separated from feather material by vacuum filtration, using synthetic polyester fibre in the filtration funnel. Extracts were placed in a 50 °C water bath and subsequently evaporated. Extract residues were reconstituted in a small volume of phosphate buffer system (PBS; 0.05M, pH 7.6) and frozen at –20 °C until analyzed by radioimmunoassay (RIA). We assessed the recovery efficiency using feather samples spiked with a small amount (approximately 5000 counts per minute) of ^{3}H-corticosterone in the extraction. Approximately 96% of the radioactivity was recoverable in the reconstituted samples. For more information about validation, see Supplementary Appendix S1 in [105].

$CORT_f$ levels were determined by RIA, and all $CORT_f$ assays were performed at the University of Saskatchewan, Canada. Measurements were performed on reconstituted methanol extracts and were duplicated. Samples were randomized throughout 10 assays that had an average (± SD) intra-assay coefficient of variation of 7.1 (± 3.03) %. Average (± SD) minimum detectability (ED80) was 11.19 (± 1.11) pg/assay tube, but we had no undetectable samples and all assay values were above this limit. Data values are expressed as pg CORT mm^{-1} of feather, which gives a valid estimate of CORT per unit time of feather growth for validation see [105,106,108].

6.2.3. Stable isotope analysis

A small (~1 mm^2) piece of feather was cut from the proximal vane of each feather prior to CORT analysis. Each feather sample was cleaned by ultrasonic shaking for 20 min in 2:1 methanol : chloroform (3 times) and in deionised water in glass vials, then dried at 70 °C. Approximately 0.5–1 mg of each feather piece was loaded into a tin cup and combusted at 1020 °C in an elemental analyzer (Carlo Erba EA1500 NC; Carlo Erba, Milan, Italy) connected to a mass spectrometer (Finnigan Delta Plus XL; Finnigan, Bremen, Germany). Stable isotope abundance was expressed in standard notation relative to V-PDB and AIR for C and N, respectively: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/ R_{standard}) - 1] \times 1000$, where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and standards, respectively. All samples were analyzed three times on different days, and two standards were measured for every ~10 samples. Based on numerous measurements of inorganic and organic international reference standards, the analytical precision of our isotope measurements is about ±0.1 ‰. The normalized scale for $\delta^{13}C$ values of the IAEA standard was used according to [297]. Three $\delta^{13}C$ and three $\delta^{15}N$ values were over three standard deviations greater than the mean and therefore omitted from analyses as outliers.

6.2.4. Statistical analyses

We first analyzed patterns of spatial associations of $CORT_f$ across the entire metapopulation using Moran's I index [298,299]. We assumed a null hypothesis of random spatial distribution of $CORT_f$ where a statistically significant positive deviation from the expected value of Moran's I would represent a higher similarity of $CORT_f$ among individuals than expected by chance alone. First, we performed a global analysis with the

entire dataset for each feather-year. We calculated all pair-wise distances between individuals, and grouped them in distance classes ("bins") with a lag of 5 km from 0 to 40 km. Due to low sample sizes, the rest of the individuals were merged into two bins (40-100 km and > 100 km) for feather-year 2004, and into one bin (> 40 km) for feather-year 2005. The number of pair-wise comparisons ranged from 244 to 3938 (2004), and 38 to 850, respectively (2005). We conducted a second analysis at small spatial scale with only individuals from feather-year 2004 and used bins with a lag of 500 m from 0 to 5 km. Spatial analyses were conducted using SAM v. 4 [300], and test significance ($\alpha = 0.05$) was calculated with 999 permutations.

We then analyzed relationships between $CORT_f$, SIs, and the physical environment by generalized linear mixed models (GLMMs) using PROC GLIMMIX in SAS v. 9.2 (SAS Institute, Cary, NC, USA). We developed sets of candidate models for each of our four predictions. We considered habitat (NIAL, INTA, NVEG, veg6), weather (PDM, $TMAX_{moult}$), SIs ($\delta^{13}C$ and $\delta^{15}N$), and spatial structure (population size, isolation, and density) variables as predictors in separate models with $CORT_f$ as the dependent variable. We then considered habitat (NIAL, INTA, NVEG, veg6) and weather (PDM, $TMAX_{moult}$) variables as predictors in separate models with either $\delta^{13}C$ and $\delta^{15}N$ as the dependent variable. For each group of candidate models, we considered all possible combinations of predictor variables, and additionally considered variables individually. All sets of candidate models additionally included feather-year as a covariate, a predictor × feather-year interaction term, and population identity as a random term. $CORT_f$ values were log transformed and $\delta^{15}N$ values were square root transformed to improve normality.

Model selection procedures were implemented to assess the strength of evidence for the relative influence of the different independent variables included in the models for each tested prediction [301,302]. We included the entire set of independent variables and covariates separately for the full models of each tested prediction. All candidate models (i.e. all possible combinations of variables) were then derived from the full models. Then, we used information-theoretic inference based on the Akaike's Information Criterion corrected for small sample sizes (AIC_c) and ranked the candidate models according to their differences in AIC_c. Candidate models within two AIC_c points of the most parsimonious model (i.e. smallest AIC_c) were considered as having similar statistical support.

6.3. Results

Overall, $CORT_f$ values were significantly higher in feather-year 2004 than they were in 2005 (mean ± SE: 2004 = 8.06 ± 0.35 pg mm^{-1}, 2005 = 6.27 ± 0.28; $F_{1,135} = 8.54$, $P = 0.004$; Fig. 6.2a). $\delta^{13}C$ values were significantly lower in 2004 than in 2005 (mean ± SE: 2004 = -22.10 ± 0.08 ‰, 2005 = -21.38 ± 0.10 ‰; $F_{1,126} = 28.60$, $P < 0.001$; Fig. 6.2b), whereas $\delta^{15}N$ values did not differ between feather-years (mean ± SE: 2004 = 4.73 ± 0.17 ‰, 2005 = 5.16 ± 0.40 ‰; $F_{1,128} = 1.46$, $P = 0.23$). PDM was greater in 2004 than in 2005 (mean ± SE: 2004 = 45.97 ± 6.10 mm, 2005 = 23.88 ± 3.41 mm; $F_{1,10} = 9.98$, $P = 0.01$; Fig. 6.2c), whereas $TMAX_{moult}$ did not differ between years (mean ± SE: 2004 = 29.68 ± 0.61 °C, 2005 = 30.06 ± 0.35 °C; $F_{1,19} = 0.21$, $P = 0.65$). PDM and $TMAX_{moult}$ were not correlated in either year (Pearson correlations; 2004: r = -0.105, $P = 0.67$, n = 19 populations; 2005: r= 0.367, P =

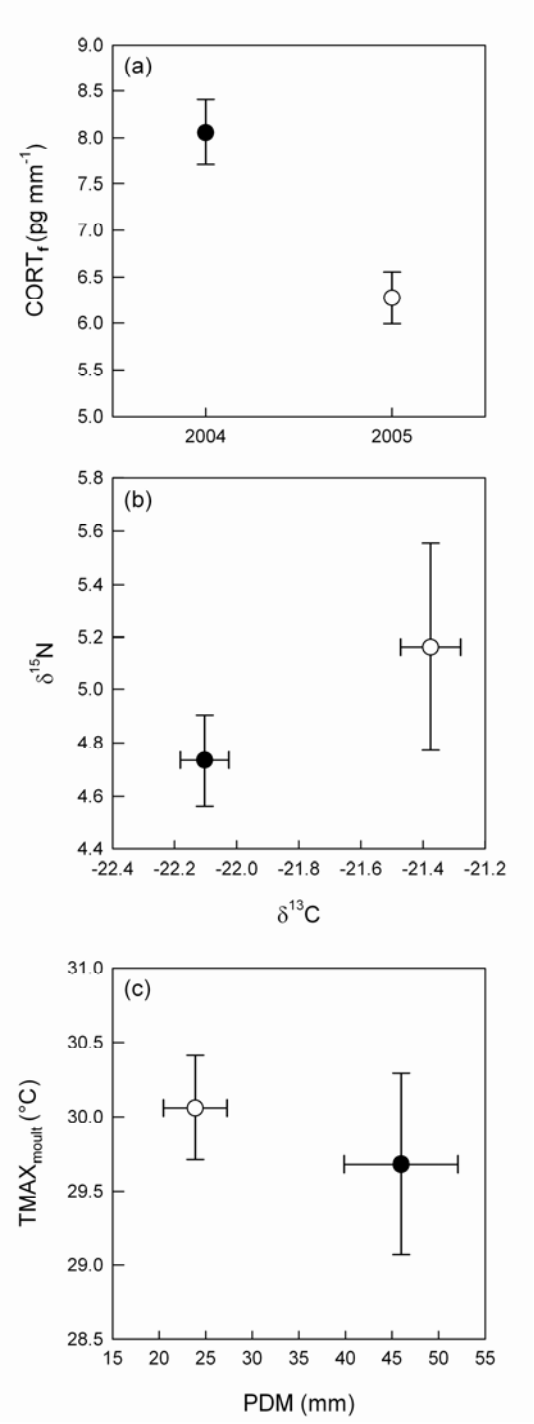


Figure 6.2. Means (± SE) plots of (a) corticosterone ($CORT_f$) and (b) $\delta^{13}C$ and $\delta^{15}N$ values from feathers of Dupont's larks, and (c) total precipitation (PDM) and average maximum daily temperature ($TMAX_{moult}$) during moulting in 2004 (filled circles) and 2005 (open circles).

0.22, n = 13 populations), suggesting that inter-annual differences in PDM operated independently of temperature.

6.3.1. Spatial patterns of $CORT_f$

Using a classic spatial autocorrelation analysis, we detected a positive effect of proximity on $CORT_f$ (Fig. 6.3). The global model for feather-year 2004 revealed statistically significant positive spatial autocorrelation only in the first bin (0 – 5 km) (expected Moran's I = -0.009, observed Moran's I = 0.195, P = 0.001). Analyses failed to detect any significant spatial effects (all P > 0.32) for feather-year 2005. At a small spatial scale we detected positive spatial autocorrelation in three bins (0 – 500 m: I = 0.506, P = 0.001; 2000 – 2500 m: I = 0.353, P = 0.002; 3000 – 3500 m: I = 0.799, P = 0.001). When we removed the largest populations (> 40 occupied territories) from this analysis, so that between-individual distances of > 3.5 km implied that individuals were in separate local populations, only the effects in the first bin were maintained (0 – 500 m: I = 0.500, P = 0.002; all other bins: P > 0.17). In summary, we found higher similarity of $CORT_f$ in pairs of individuals at very limited spatial scale and, importantly, only within discrete local populations.

6.3.2. Relationships between $CORT_f$, SIs, and the physical environment

Of the set of candidate models explaining variation in $CORT_f$ in terms of traditional habitat measures and weather during the moulting period, the most parsimonious models in each group included only feather-year (Table 6.2). This indicates that neither traditional

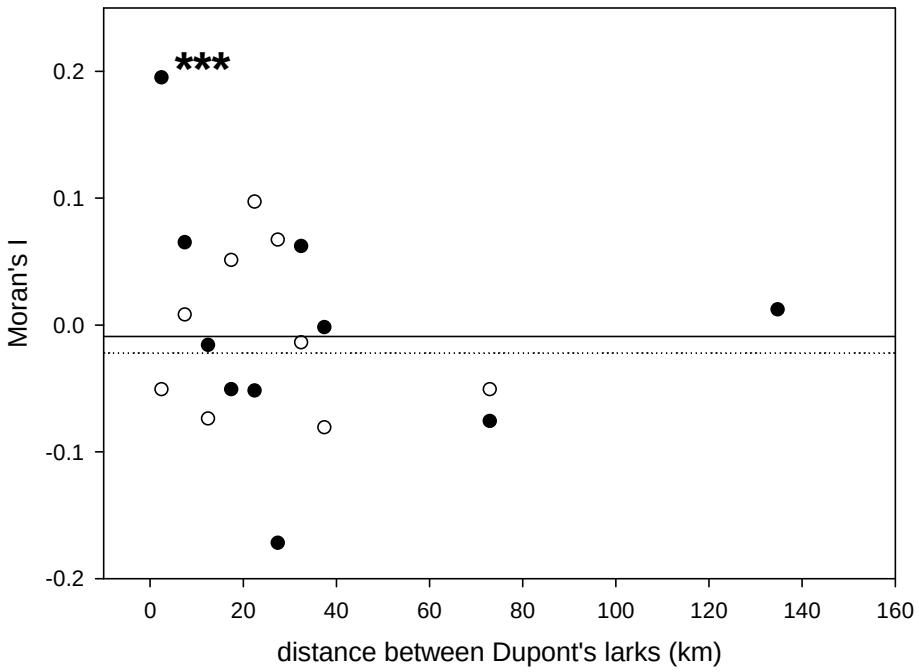


Figure 6.3. Correlogram demonstrating the geographic structure of $CORT_f$ similarity. Only pairs of individuals separated by straight-line distances less than 5 km in 2004 (marked with asterisks) showed significantly similar values ($P = 0.001$). Filled and open circles represent values for 2004 and 2005, respectively. Lines (solid: 2004; dotted: 2005) show expected values of Moran's I.

Table 6.2: Best supported models, ranked by AIC_c value, explaining variation in lark $CORT_f$ in terms of traditional habitat variables, weather during the moulting period, and $\delta^{15}N$ and $\delta^{13}C$. Models within 6 AIC_c points of the top model are shown for each group. See text for definitions of variables.

Group	Model	Term	estimate	SE	d.f.	t	P	AIC_c	ΔAIC_c
Habitat	1	feather-year	0.08	0.029	121	2.79	0.006	-110.15	0
	2	intercept	0.81	0.023	21	35.07	< 0.0001	-107.78	2.37
	3	veg6	-0.01	0.014	121	-0.82	0.41	-104.07	6.08
		feather-year	0.08	0.029	121	2.83	0.006		
Weather	1	feather-year	0.08	0.029	121	2.79	0.006	-110.15	0
	2	intercept	0.81	0.023	21	35.07	< 0.0001	-107.78	2.37
	3	PDM	0.003	0.001	121	3.12	0.002	-105.06	5.09
Isotopes	1	$\delta^{13}C$	0.03	0.036	119	0.75	0.46	-120.21	0
		feather-year	-2.39	0.84	119	-2.84	0.005		
		$\delta^{13}C$*feather-year	-0.11	0.039	119	-2.89	0.005		
	2	$\delta^{13}C$	-0.07	0.016	121	-4.49	< 0.0001	-120.15	0.06
	3	$\delta^{13}C$	-0.06	0.017	120	-3.67	0.0004	-116.69	3.52
		feather-year	0.039	0.03	120	1.32	0.19		

habitat measures nor weather did a better job than feather-year at explaining the variation in $CORT_f$. By contrast, when we considered relationships with isotopes, the best-supported model included $\delta^{13}C$, feather-year, and a significant interaction term (Table 6.2). When we analyzed the relationship between $\delta^{13}C$ and $CORT_f$ separately by feather-year, we found a significant negative relationship for 2004 ($F_{1,81} = 17.24$, $P < 0.0001$), but not for 2005 ($F_{1,27} = 0.60$, $P = 0.44$). Our sample size was considerably smaller in 2005, which likely reduced statistical power. The next best supported model had nearly identical statistical support (Table 6.2), included only $\delta^{13}C$, and revealed the same negative relationship. These results indicate that overall $CORT_f$ appears to negatively covary with $\delta^{13}C$. There was no statistical support that any model including $\delta^{15}N$ explained significant variation in $CORT_f$.

Of the models explaining variation in $\delta^{13}C$ in terms of habitat, the best-supported included only feather-year (Table 6.3). The next most parsimonious model received nearly identical statistical support and also included INTA and NVEG (Table 6.3), suggesting that both these landscape matrix variables were related to $CORT_f$. Of the models explaining variation in $\delta^{13}C$ in terms of weather during moulting, the most parsimonious included effects of $TMAX_{moult}$ and feather-year, but the estimate on the former parameter was poor (Table 6.4). A model receiving nearly identical statistical support included only feather-year (Table 6.4). Thus it appears that the influence of weather on $\delta^{13}C$ was likely very weak.

The best supported models explaining variation in $\delta^{15}N$ in terms of habitat and weather included only the intercept (Table 6.5). Although similar statistical support was found for a model including NVEG only (Table 6.5), this model still nearly completely lacked explanatory power. Thus, neither habitat nor weather during moulting explained significant variation in $\delta^{15}N$.

Table 6.3: Best supported models, ranked by AIC_c value, explaining variation in $\delta^{13}C$ from lark feathers in terms of traditional habitat variables. Models within 6 AIC_c points of the best-supported model are shown for each group. See text for definitions of variables.

Model	**Term**	**estimate**	**SE**	**d.f.**	**t**	**P**	**AIC_c**	**ΔAIC_c**
1	feather-year	-0.63	0.13	121	-4.91	< 0.0001	319.86	0
2	INTA	-0.05	0.017	119	-3.13	0.002	319.99	0.13
	NVEG	-0.05	0.015	119	-3.47	0.0007		
	feather-year	-0.62	0.13	119	-4.93	< 0.0001		
3	NIAL	0.03	0.012	119	2.47	0.02	324.46	4.6
	INTA	-0.01	0.02	119	-0.7	0.49		
	feather-year	-0.62	0.13	119	-4.89	< 0.0001		
4	NIAL	-0.03	0.03	118	-0.97	0.34	324.51	4.65
	INTA	-0.08	0.04	118	-2.37	0.02		
	NVEG	-0.08	0.04	118	-2.29	0.02		
	feather-year	-0.63	0.13	118	-4.98	< 0.0001		
5	NIAL	0.03	0.014	119	2.06	0.04	324.79	4.93
	NVEG	-0.008	0.02	119	-0.41	0.69		
	feather-year	-0.61	0.13	119	-4.79	< 0.0001		

Table 6.4: Best supported models, ranked by AIC_c value, explaining variation in $\delta^{13}C$ from lark feathers in terms of weather during the moulting period. Models within 6 AIC_c points of the best-supported model are shown for each group. See text for definitions of variables.

Model	**Term**	**estimate**	**SE**	**d.f.**	**t**	**P**	**AIC_c**	**ΔAIC_c**
1	$TMAX_{moult}$	-0.17	0.095	120	-1.81	0.07	319.51	0
	feather-year	-0.6	0.13	120	-4.67	< 0.0001		
2	feather-year	-0.63	0.13	121	-4.91	< 0.0001	319.86	0.35
3	$TMAX_{moult}$	-0.08	0.17	119	-0.45	0.65	320.34	0.83
	feather-year	3.77	6.35	119	0.59	0.55		
	$TMAX_{moult}$*feather-year	-0.14	0.21	119	-0.69	0.49		

Table 6.5: Best supported models, ranked by AIC_c value, explaining variation in $\delta^{15}N$ from lark feathers in terms of traditional habitat variables and weather during the moulting period. Models within 6 AIC_c points of the best-supported model are shown for each group. See text for definitions of variables.

Group	**Model**	**Term**	**estimate**	**SE**	**d.f.**	**t**	**P**	**AIC_c**	**ΔAIC_c**
Habitat	1	intercept	2.27	0.093	21	24.47	< 0.0001	148.23	0
	2	NVEG	-0.03	0.011	121	-2.76	0.007	148.68	0.45
	3	feather-year	-0.039	0.069	121	-0.56	0.58	151.44	3.21
	4	Veg6	-0.023	0.058	122	-0.39	0.7	151.96	3.73
	5	NIAL	0.014	0.008	121	1.67	0.1	153.25	5.02
Weather	1	intercept	2.27	0.093	21	24.47	< 0.0001	148.23	0
	2	feather-year	-0.039	0.069	121	-0.56	0.58	151.44	3.21
	3	$TMAX_{moult}$	0.025	0.056	121	0.45	0.66	151.97	3.74

6.3.3. Relationships between $CORT_f$ and the spatial structure of the metapopulation

The best-supported models explaining $CORT_f$ in terms of population size, isolation, and density included only feather-year (Table 6.6). Population size, isolation, and density variables failed to explain more variation than the intercept alone. Thus, we lack statistical evidence that any of these variables were significantly related to $CORT_f$.

6.4. Discussion

6.4.1. Interplay among $CORT_f$, SIs, and the physical environment

Our results indicate that synchronising $CORT_f$ with SIs is a promising approach to studying habitat-physiology relationships in birds. Surprisingly, and contrary to our prediction, $CORT_f$ was not related to any of the traditional habitat variables we measured. However, by using SIs as a proxy for the local environment, we detected a significant negative relationship between $\delta^{13}C$ and $CORT_f$. The direction of this relationship is contrary to what we predicted and suggests that larks feeding on diets that were relatively depleted in ^{13}C may have been coping with relatively greater energetic challenges. Although our study cannot determine conclusively the factor(s) that caused variation in $CORT_f$ or $\delta^{13}C$, the fact that these variables covaried suggests that larks were responding physiologically to some aspect of their local environment to which $\delta^{13}C$ was apparently sensitive. Values of $\delta^{13}C$ were not high enough to indicate that larks in our study were feeding from a C_4-based food web [278], but lark feathers were significantly enriched in ^{13}C

in 2005 relative to 2004. Diet composition can significantly affect $\delta^{13}C$ values [303-305] and blood levels of

Table 6.6: Best supported models, ranked by AIC_c value, explaining variation in lark $CORT_f$ in terms of population size, isolation, and density. Models within 6 AIC_c points of the best-supported model are shown for each group. See text for definitions of variables.

Model	Term	estimate	SE	d.f.	t	P	AIC_c	ΔAIC_c
1	feather-year	0.08	0.029	121	2.79	0.006	-110.15	0
2	intercept	0.81	0.023	21	35.07	< 0.0001	-107.78	2.37
1	feather-year	0.08	0.029	121	2.79	0.006	-110.15	0
2	intercept	0.81	0.023	21	35.07	< 0.0001	-107.78	2.37
3	isolation	-0.05	0.05	120	-1.12	0.26	-107.11	3.04
	feather-year	0.08	0.029	120	2.64	0.009		
1	feather-year	0.08	0.029	121	2.79	0.006	-110.15	0
2	intercept	0.81	0.023	21	35.07	< 0.0001	-107.78	2.37
3	density	-0.01	0.23	121	-0.05	0.96	-106.66	3.49
4	density	-0.047	0.23	120	-0.21	0.84	-105.06	5.09
	feather-year	0.08	0.03	120	2.79	0.006		

Group	Population size	Isolation	Density

CORT [164,306]. Thus, it is likely that $\delta^{13}C$ values in our study reflected the quality or type of food that larks consumed (e.g., an abundance of C_4-feeding insects in 2005), which may have affected individual physiology. Values of $\delta^{13}C$ were related, to a limited extent, to the land uses surrounding lark habitat patches (i.e., in the landscape matrix), but not vegetation type within patches. This result is in line with previous findings that lark occurrence in our study area is related to land uses surrounding, but not within, lark habitat patches [280]. Larks forage exclusively within habitat patches [285,286] and were likely influenced by diet subsidies originating beyond the local environment [307]. Thus, our study highlights the importance of considering physiology in a landscape context, and shows how combining $CORT_f$ with $\delta^{13}C$ can help provide that perspective.

We did not detect relationships between $CORT_f$ and either $\delta^{15}N$ or weather during the moulting period, despite considerable variation in both. Moreover, contrary to our prediction, we only found weak support that land use influenced $\delta^{15}N$ values. Values of $\delta^{15}N$ did not differ significantly between years; however, there was substantial variability among individuals (1-12‰). Larks are virtually imprisoned in suitable habitat patches [280] and their diet likely comprises a large range of available arthropod prey [285,286]. Signatures of nitrogen isotopes would therefore be expected to vary with food items that were opportunistically available and locally abundant, and may have been additionally

influenced by the presence of nitrogen-fixing plants. Our results suggest that whatever the factor(s) responsible for variation in $\delta^{15}N$, it apparently did not significantly affect $CORT_f$. Similarly, despite a nearly two-fold decrease in PDM between 2004 and 2005, we detected no significant relationships between weather and $CORT_f$. Precipitation during the lark moulting period in our study area falls mainly as short-duration downpours [295], and PDM was measured over three months. If the timing of individual moult did not coincide well with periods of rainfall, then $CORT_f$ would not have been strongly influenced by responses to precipitation. Alternatively, larks may have been within their physiological ability to cope with the variation in weather they experienced.

6.4.2. $CORT_f$ and the spatial structure of the metapopulation

Why was none of the habitat and spatial structure variables related to $CORT_f$? Spatial autocorrelation analysis revealed that, for the same inter-individual distance, larks within suitable habitat showed significant similarity in $CORT_f$, whereas individuals separated by unsuitable habitat did not. In other words, the closer individuals were spatially, the more similar their $CORT_f$ values were, but only within local populations. We reason that this is physiological evidence of habitat change. Habitat loss and fragmentation has led to highly isolated and island-like local populations in a predominantly unsuitable landscape [see also 308]. Consequently, the composition of the landscape and its spatial configuration has undergone large changes, and $CORT_f$ patterns that represent physiological responses to local conditions may no longer be correlated. Additionally, inbreeding and genetic relatedness of larks increase with increasing habitat patch isolation

in our study area [309]. This suggests an intriguing explanation that patterns of $CORT_f$ could indicate genetic relatedness among spatially closer individuals. Future research should clarify the relative influence of fragmentation and inbreeding on patterns of $CORT_f$.

It is possible that the habitat and spatial structure variables we measured were not relevant to lark physiology, or were operating at spatial and temporal scales that made relationships between these variables and $CORT_f$ unlikely. Larks are confined to remnant habitat patches and local populations have independent population dynamics [280,289]. Therefore, factors that operate beyond the patch environment (e.g., isolation) may not have any influence on lark physiology, which is determined at a local scale. Although this is a reasonable explanation, when we considered a more appropriate scale and variables that should directly influence physiology (i.e., local vegetation community, size of local populations), we still failed to find relationships with $CORT_f$. Similarly, we failed to detect a relationship between density and $CORT_f$. The short time lag between when density was measured and when larks grew feathers could account for this. Although territoriality may be relaxed during moulting, the number of individuals within patches would have remained similar. Thus, we believe our measures of density were valid during the moulting period. We therefore lack evidence to support an influence of conspecific density on $CORT_f$, and add to a very small number of studies addressing this in the wild [e.g., 310].

Collectively, these are important negative results that we believe highlight a disconnection between habitat and physiology in our lark metapopulation. Although patch- and landscape-level variables such as patch size, isolation, and composition of the landscape matrix may influence population dynamics in highly fragmented populations, we

have shown that individual physiology is likely determined at much smaller spatial scales and may operate entirely separately from these processes.

6.4.3. Concluding remarks

Our study highlights the utility of combining feather-based measures of CORT and SIs to study habitat-GC relationships. Had we relied solely on traditional measures of habitat, and even those measured at the patch level, we would have erroneously concluded that habitat was not influencing $CORT_f$ in larks. It is crucial to avoid committing this kind of error when managing any population, but especially so for endangered species, and our feather-based approach may help reduce the likelihood of such mistakes. Importantly, we show that measures of habitat and population structure likely to influence population dynamics in larks were not related to an integrated measure of CORT physiology. We see this as evidence of the disconnection between habitat and physiology in highly fragmented populations. Our approach helped us better understand habitat-physiology relationships at the individual level. $CORT_f$ was related to $\delta^{13}C$, providing evidence of a physiological response to the local ecological conditions to which larks were exposed. Future studies will benefit from measuring SI signatures in local vegetation and prey items to better understand SI signatures in feathers. Additionally, consideration of genetic effects, particularly in populations with limited dispersal, may offer further insight into spatial variation in $CORT_f$. In conclusion, our non-invasive feather-based approach is ideal for use in the field and can provide powerful ecophysiological information to habitat studies of birds.

Chapter 7. General discussion

7.1. Overview

I detected relationships between feather CORT and ecological variables in every context I studied. The long-term integrated measure was related to a diversity of challenges including rearing environment, habitat quality, and reproduction. The effects of all these factors operated over time periods longer than one week. This suggests that feather CORT quantified activation of the HPA axis in response to ecological variability in general, rather than in response any specific type(s) of challenges. A unifying theme of my work was that, when interpreted in the proper context, feather CORT was related to energetic demand or exertion. This indicates that feather CORT may be a proxy for individual energetics. My work suggests that feather CORT will likely be useful in moving both theoretical and applied research towards a holistic, energetics-based perspective of GC ecophysiology.

7.2. Project comparisons

Nestling swallows and shearwaters that experienced more challenging conditions tended to have feather CORT values that were on average ≤ 1 pg/mm different than control birds (Table 7.1, Chapters 2 and 3). Although the magnitude of responses appeared to be similar between the two species, the direction of responses differed, suggesting different biological effects. This difference in direction may have been due to the type of challenge

experienced by each species. Tree swallows were likely coping with variability in microclimate, which would require energetic expenditure (i.e., increased CORT secretion).

Table 7.1. Comparison of feather CORT values (pg/mm) from my thesis projects.

Source	Species and age	Context	Year	Min	Max	Range	Mean	SE
Chapter 2	Nestling tree swallows	Hatched and raised in aspen	2009	1.69	6.08	4.39	3.17	0.17
		Hatched in aspen, raised in plywood		1.79	5.89	4.10	3.36	0.17
		Hatched in plywood, raised in aspen		2.11	6.19	4.08	3.51	0.17
		Hatched and raised in plywood		2.08	7.71	5.63	3.78	0.23
Chapter 3	Nestling Cory's shearwaters	Raised by handicapped parents	2004	2.96	5.92	2.96	4.45	0.83
		Raised by unmanipulated parents		3.45	8.10	4.65	5.46	1.61
Chapter 4	Captive adult Clark's nutcrackers [6]	Experimentally enriched cages	2007	1.53	27.49	25.96	9.52	1.68
		Non-enriched controls		1.81	15.11	13.30	6.11	0.69
		Experimentally enriched cages	2008-09	3.46	27.42	24.46	7.61	0.68
		Non-enriched		3.60	24.50	20.89	7.56	0.54

[6] Data presented here do not include final section of second feather.

Source	Species and age	Context	Year	Min	Max	Range	Mean	SE
Chapter 5	Adult tree swallows	Variation in reproductive effort	2009	1.65	3.45	1.80	2.44	0.10
Chapter 6	Adult Dupont's larks	Variation in physical environment and spatial structure of population	2004	3.44	21.09	17.65	8.06	0.35
			2005	3.27	13.61	10.34	6.27	0.28

By contrast, shearwaters were coping with a dwindling energy supply, which would necessitate a strategy to conserve energy (i.e., suppressed CORT secretion). Life history variation may provide some additional insight into species-specific responses. Shearwater chicks may have adaptively suppressed CORT secretion because their extended nestling period allowed for compensatory growth. By contrast, tree swallows may not have been willing or able to risk slowed growth associated with CORT suppression [35] because they have a relatively short nestling period. Interestingly, survival to fledging was not affected in either species, suggesting that individuals were within their ability to cope physiologically with energetic challenges.

Compared to nestlings, there was considerably more variation in the range of individual adult feather CORT values (Table 7.1, Chapters 4, 5, and 6). In part, this likely reflects species differences, but could also reflect the functioning of the HPA axis, which would be fully mature in adults. If the range of values is any indication of the strength of responses, then the greatest feather CORT responses were evoked by enrichment and its removal in nutcrackers (Chapter 4). Birds receiving experimental enrichment showed CORT values nearly double that of unenriched controls, but these changes were apparently not related to behavioural changes. This is an interesting finding considering that enrichment appeared to be the least energetically-demanding challenge I studied. The enrichment study differed significantly from the others in my thesis in two ways. First, it was the only study conducted with captive birds and this may have influenced the sensitivity of birds to challenges. Second, the change of environment at such a small scale may have been a predominantly psychological challenge. Challenges in my other studies may have had a psychological component but were likely more physically or

physiologically demanding (e.g., reproduction, decreased provisioning). Physically demanding challenges would require increased energy and CORT would facilitate this [15]. The psychological context of challenges can influence HPA activity [89], so it is possible that the perceived challenge of enrichment objects influenced CORT levels in nutcrackers. It is not clear if the percent change in CORT in response to the (presumably) psychological challenge of enrichment indicates an increased level of energy demand relative to more physical challenges. Recent work suggests that psychological challenges may not incur an actual increase in energy demand [311], but this need not preclude preparative psychological activation of the system.

Larks also showed substantial variation in feather CORT (Chapter 6). Lark feather CORT was related only to $\delta^{13}C$, which was a proxy for habitat. Interestingly, it appears that the quality of habitat, rather than the amount, was the component to which larks were responding. Habitat in the Ebro Valley is degraded relative habitat in other locations throughout the lark's range. Comparisons of feather CORT from larks in better quality and more intact habitat (e.g., Morocco) would provide some context for the range of values observed in my study.

The range of feather CORT values for adult tree swallows (Chapter 5) was considerably smaller than it was for other species, which is interesting considering that reproduction is an energetically-demanding activity. Evidence suggests that tree swallows may be able to raise broods of up to seven or eight without any major costs [260], so perhaps the range of swallow CORT values reflect this. Quality may also play a role, as I suggest in Chapter 5, because the birds sampled included only those that survived to reproduce the subsequent year. This could also be a sample size issue; a wider sample of

birds, including those that failed to reproduce and those that were most productive, would be expected to express a wider range of CORT values.

Cross-species comparisons should be interpreted cautiously, particularly among different ages and challenges. However, compared to previously published studies using feather CORT [64,105,107], my results are within the ranges of values reported and are even on the low end. All variables that I related to feather CORT persisted for a minimum of 10 days. Although it is not possible to know exactly how long the challenge lasted in each of these cases, these variables are likely more representative of longer-term ecological variation than acute stressors. The significant relationships between these challenges and feather CORT suggests that a long-term integrated measure of CORT was appropriate for measuring HPA responses to these factors.

7.3 Energetics: towards a holistic perspective of CORT ecophysiology

A unifying theme of my findings is that, when considered in an appropriate ecological context, feather CORT predictability could be conceptually linked to actual or perceived energetic demand or exertion. Energy is a common currency of both physiological and ecological systems. Therefore, it is not surprising that a hormone integral to energy partitioning is related to diversity of ecological, behavioural, and physiological factors, as my work and that of numerous others suggests. Clearly, CORT does not act alone in this regard [13,142]. However, the sensitivity of the long-term integrated measure to a variety of ecological stimuli suggests that CORT is integral to how individuals interface physiologically with their environment. By mobilizing energy substrates, CORT helps

individuals adjust available energy appropriately to cope with whatever demands the environment—both internal and external—may place upon them [14-16]. However, rather than measuring the instantaneous CORT response to those demands, feather CORT measures the total response over the period of feather growth (i.e., the integrated response).

Energy partitioning may be a common thread that explains how feather CORT relates to ecophysiological variation across different contexts. Thus, I suggest that feather CORT measures an energetic correlate of ecophysiological variation in birds. In this context, levels of GCs may be an indicator of demand for specific types (i.e., carbohydrate-, protein-, or lipid-based) or amounts of energy or, depending on when GCs are measured, a proxy for energetic expenditure. I am not suggesting that this is a new idea; numerous authors frame their GC results and models in the context of energy, and entire models are built on the concept (e.g., [8,29,71,252,312]). However, as my work shows, feather CORT can be linked conceptually to individual energetics.

To be robust, an energetics explanation should have broader explanatory power. For example, why are baseline CORT levels higher in species where the importance of current reproduction is presumed to be higher [27,313]? An energetics explanation would suggest that relatively more energy is directed to reproduction when the perceived value is higher. As another example, an energetics explanation would predict a relationship between body size and CORT because the lower mass-specific metabolic rate and lower rate of heat loss of larger endotherms [314] should require less CORT relative to smaller individuals. Indeed, such a relationship between body size and CORT exists [27]. Furthermore, metabolic rates are generally slower for sedentary tropical species than they

are for migratory temperate ones [315], and daily energy expenditure is higher in arctic breeding birds than in temperate ones [316]. This suggests that CORT should be lower in lower latitude species than in higher latitude ones. Evidence suggests this is the case, at least for maximum CORT levels [27]. Breeding season length is believed to be a major driver of temperate-tropical CORT differences in birds [29]. Although I agree with this interpretation, an energetics explanation suggests an additional overlapping explanation. Latitudinal patterns of energy availability [317] may explain why individuals living in tropical environments do not need to exert as much energy as their temperate counterparts. Daily energy expenditure is driven in part by temperature and food [318,319]. Birds living in places that are more energy-rich (more food and warmth) should have lower CORT, and data support this idea [29,320].

The above evidence, in conjunction with my thesis studies, provides examples of how energetics may provide a useful framework for predicting patterns of CORT within and across ecological contexts. But how do feather CORT values reflect the complex energetics generated by continuous responses to ecological variability? Moreover, can the energetics concept be extended to explain how CORT values relate to fitness?

7.4. Future outlook

7.4.1. The ecophysiological niche model

CORT values are governed by physiological regulators [142] that respond to the myriad stimuli that contribute to energy balance (e.g., body condition, territorial defence,

ambient temperature). I argue that feather CORT, as an integrated measure, reflects to a certain extent the individual's "solution" to resolving, in a way that maximizes fitness, the numerous perceived and actual energetic demands varying throughout space and time during feather growth. In a sense, this is similar to the allostasis concept [8] in that it relates CORT to an energetic currency that reflects the energetic state of the individual. Here, I develop a theoretical model that differs from allostasis in that it does not attach any physiological outcome or state to levels of CORT. Rather, it seeks to explain how a long-term integrated measure relates to variation in CORT physiology in the broadest ecophysiological sense. The theoretical model I am introducing considers two axes which reflect the natural range of environmental and physiological variation that directly or indirectly influence individual energy balance throughout time. I use feather CORT as the physiological measure, but the model accommodates any integrated physiological variable relevant to energy balance.

Starting from the Reactive Scope Model [142], which expresses a flexible range of physiology throughout time (Fig. 7.1), I add an environmental axis to explain what is implicit in both that model and allostasis: physiological mediators reflect responses to the environment over time. This sets physiology in an environmental context in which CORT values must be interpreted. The environmental axis represents the natural

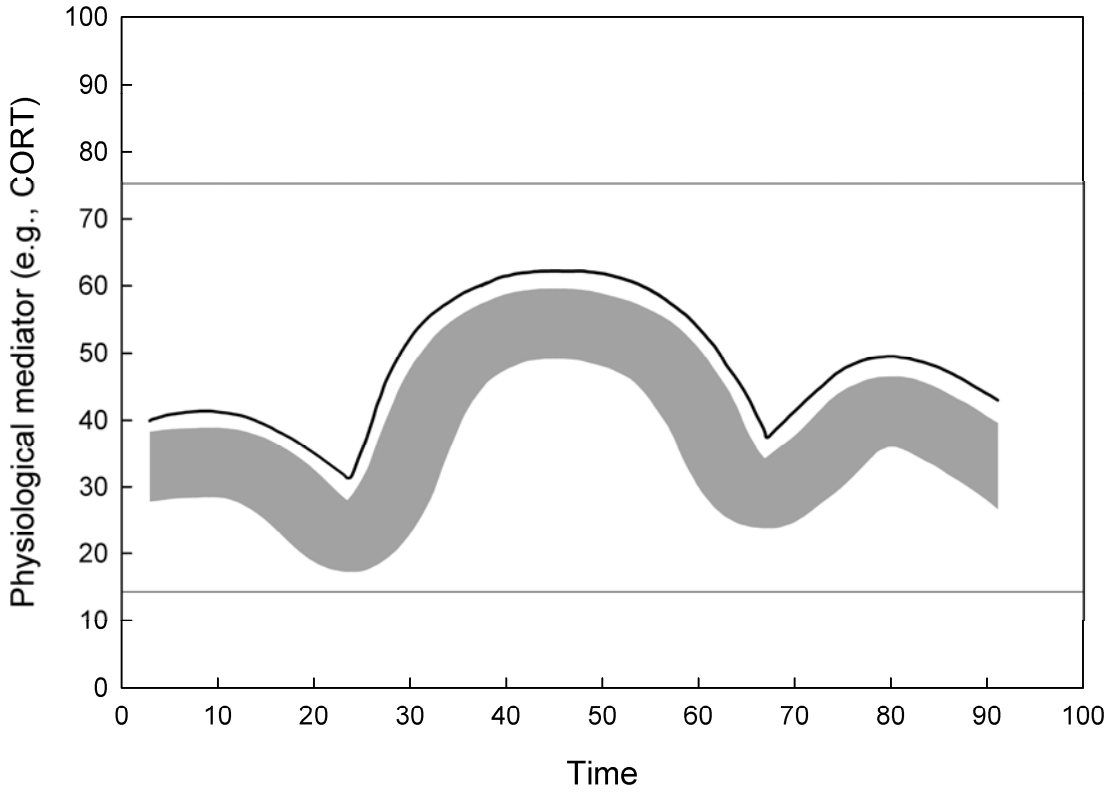


Figure 7.1. Depiction of a hypothetical range of values of physiological mediator (e.g., CORT) expressed by an individual over time. Grey bar represents circadian variation in physiology; black line immediately above grey bar represents peak values in response to predictable daily challenges (e.g., foraging). Horizontal lines define the range of possible values in a healthy animal. Scales of both axes are arbitrary. Figure adapted from [142].

range of variability over time of all biotic and abiotic components of the environment external to the individual. For simplicity I consider this as a single axis; in reality it is a multi-dimensional abstraction (Fig. 7.2 “a”) that represents numerous interacting environmental parameters (e.g., climate, vegetation, conspecifics, predators). Similarly, the physiological axis represents the natural range of variability of individual physiology and behaviour. This is also a multi-dimensional abstraction (Fig. 7.2 “b”), representing innumerable interacting physiological and behavioural parameters occurring in time (e.g., immune response, growth, reproduction), that I consider as a single axis for simplicity. Because the model includes a time axis, it recognizes that both the environment and individual physiology and behaviour can vary on a short-term basis (i.e., the context of an immediate stressor) and over longer time periods (e.g., the context of seasonal changes).

There is important overlap between the environmental and physiological axes that defines a “hypervolume” (Figure 7.3 “c”), to borrow from Hutchinson [321]. This hypervolume expresses the theoretical boundaries, for a given time and space, of an individual’s total CORT secretion that will make positive (or neutral) contributions to fitness; CORT values that fall outside this space contribute negatively to fitness. Thus, the exact shape of the hypervolume is determined by the theoretical range of CORT values that maximizes an individual’s fitness in response to all physiological and behavioural demands in time and space. This is entirely dependent on individual ecophysiological context but, all else being equal, the greater the hypervolume the greater the predicted range of CORT values that should positively contribute to fitness. In a sense, this is analogous to Romero et al.’s [89] “normal reactive scope”, but with an explicit environmental context.

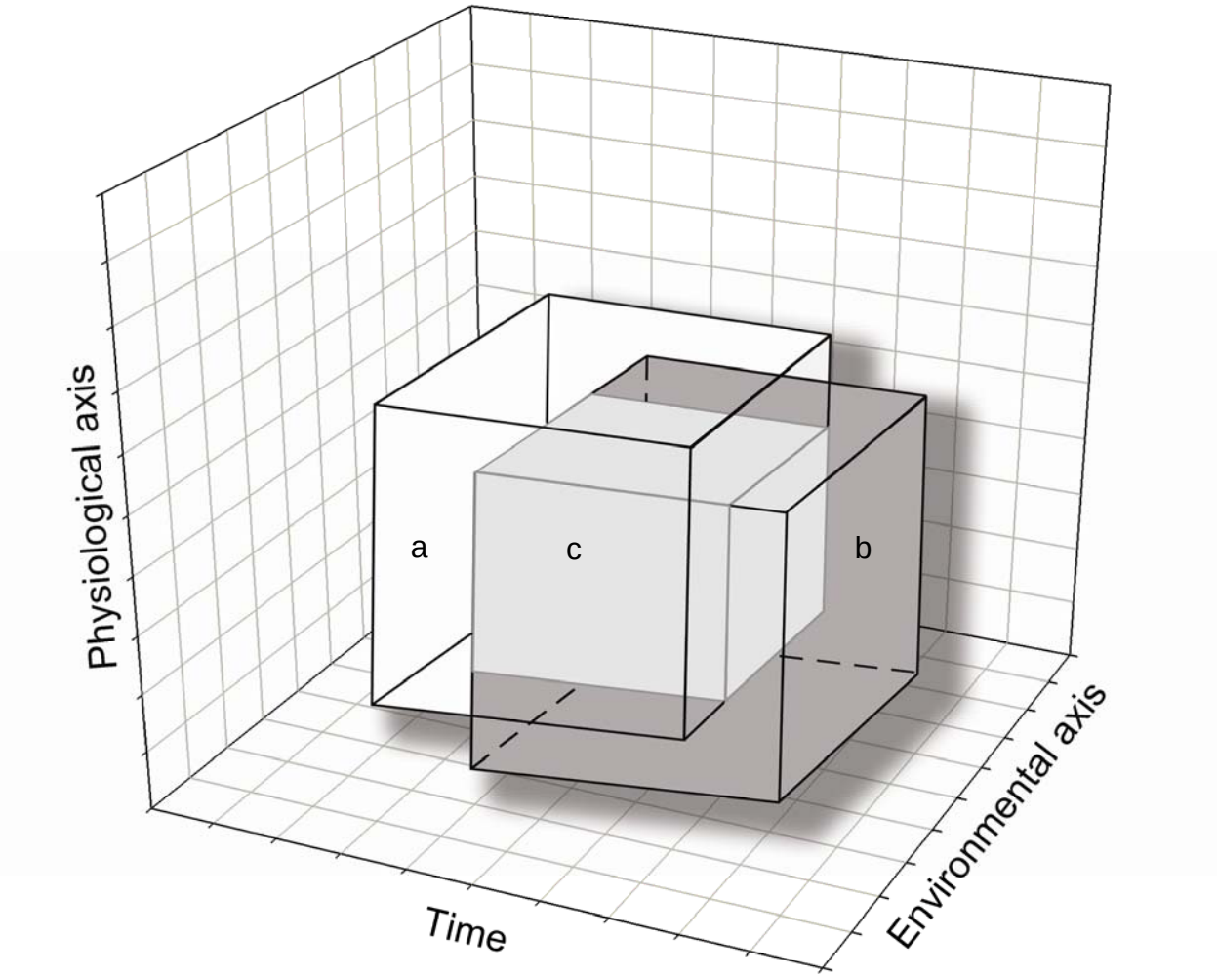


Figure 7.2. The intersection of complex sets of environmental ("a", white) and physiological ("b", dark grey) parameters related to individual energy balance over time defines an "ecophysiological hypervolume" ("c", light grey). Both the environmental and physiological axes are multi-dimensional abstractions, depicted here as three-dimensional objects for simplicity.

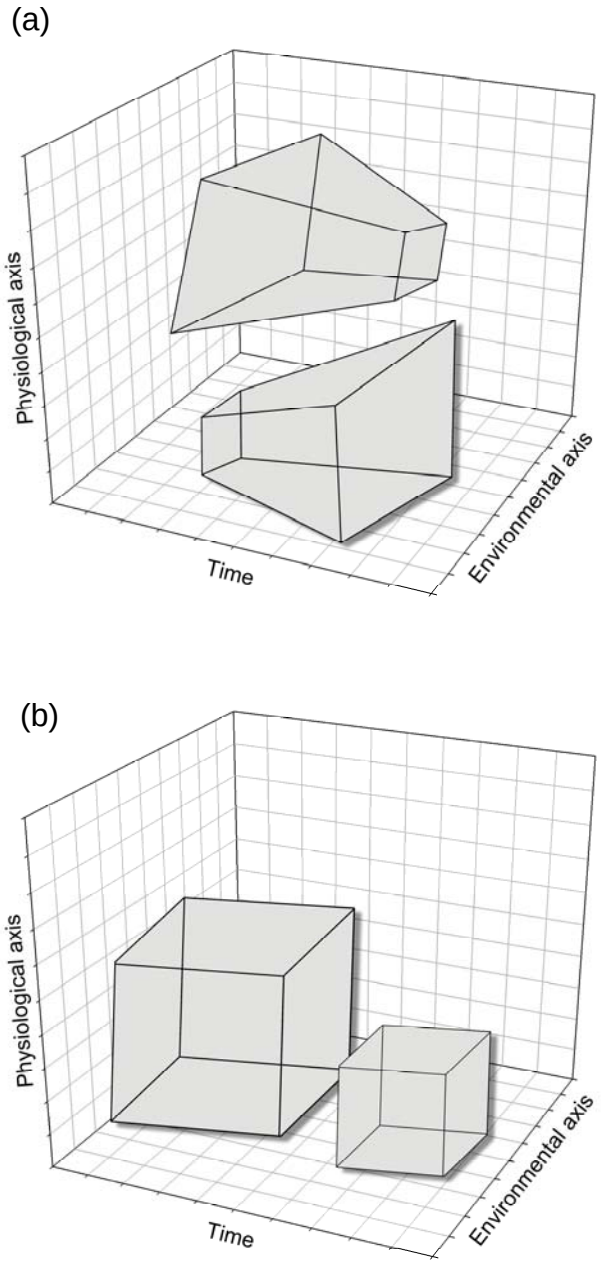


Figure 7.3. Hypothetical depictions of how individual constraints and quality could alter the shape of the ecophysiological hypervolume. (a) Ecological and physiological constraints could restrict the hypervolume early (bottom) and later (top) in life. (b) Better quality individuals (left) would be expected to tolerate a wider range of, e.g., CORT values than lower quality individuals (right), hence the enlarged hypervolume.

The dimensions of the hypervolume can change with individual characteristics such as life history stage, age, and individual quality; similarly, temporal changes in the environment can influence the range of physiological values that will positively contribute to fitness. For example, an individual that is constrained in its ability to respond to challenges, such as a nest-bound nestling with a developing HPA axis (e.g., [116]) or an aging individual (e.g., [322]) that can no longer move great distances, would be expected to have an increasing or decreasing hypervolume, respectively (Fig. 7.3a). Similarly, individual quality should influence hypervolume such that, all else being equal, better quality individuals should have a greater hypervolume (Fig. 7.3b) because they can withstand the effects of a greater range of CORT, particularly increased levels (e.g., Chapter 5).

When considered in the above terms, this hypervolume can be thought of as defining an "ecophysiological niche". This theoretical construct reflects the complexities of both the environment and the individual's physiology and behaviour throughout time and space. Feather CORT, as an integrated value, measures a portion of this hypervolume and therefore may help quantify the ecophysiological niche. That is, feather CORT measures total CORT released in response to the complex ecophysiology occurring during the period of feather growth. This may be a small or large potion of the ecophysiological niche, depending on the time scale considered. Feather CORT may therefore be useful in a way similar to how stable isotope ratios are currently being used (e.g., [323]). Combining the two may be particularly powerful, as my results suggest (Chapter 6), because it integrates information from several environmental and physiological sources. As a note of caution, CORT physiology is only a part of the greater physiological response to environmental

challenges, as Romero et al. [142] point out. Nevertheless, by measuring a multi-dimensional perspective of CORT physiology, feather CORT provides a measure better suited to studying physiological responses to complex ecological phenomena.

7.4.2. Management and conservation implications

My work provides information directly relevant to management and conservation. For example, insight into the influence of nest box microclimate on CORT in nestling tree swallows may help improve nest box design [324]. Augmenting populations of cavity nesters using nest boxes is a conservation priority for some species (for a review see [325]), and it is important to understand how nest box characteristics influence inhabitants [324]. My research adds an important physiological dimension to consider. By increasing our understanding of how some nestling Procellariids cope with decreasing food supply, my work may help efforts to mitigate effects of declining fish stocks on seabirds. My results suggest that lower (rather than the often expected higher) CORT values indicate birds coping with nutritional challenges. Combining CORT with SIs from Dupont's lark feathers provided a physiological interpretation of the ecological conditions to which larks were exposed. Lark physiology was influenced more by factors at a local scale than at scales that drive population processes. This information is directly relevant to how populations of this endangered habitat specialist are managed.

The evidence above suggests that feather CORT can provide a measure of how individuals are generally coping with their environment. As such, feather CORT may be a valuable biomarker [272]. Feathers are easy to collect and store and are suitable for use in

conservation contexts in the wild or captivity. Importantly, because feathers travel with their bearer, it is possible to collect them at any time between moults. This is particularly useful for species that are secretive or otherwise inaccessible during part of their life, but are more accessible or aggregate at other times. By combining this information with other metrics (e.g., SIs), we may achieve a more complete understanding of how individuals perceive and respond to their environment. This information is essential to proper management and conservation plans. Moreover, using feather CORT in a CORT-energetics framework may be especially useful in helping understand avian responses to climate change.

7.4.3. Future research needs

We can more easily understand the consequences CORT has for measures of performance and components of fitness if we can find a commonality in how we interpret the causes of variation in CORT. Without this connection, a reductionist approach will continue to highlight disjointed pieces of a complex ecophysiological puzzle. The intent of my thesis was to gain an understanding of a measure of CORT physiology that moves beyond "baseline" and "stress-induced" values. I did this by addressing the causes of variation in feather CORT in the context of longer-term challenges. My research suggests that energetics can provide a unifying framework within which to interpret CORT levels.

Future research will be instrumental in gaining a fuller understanding of how feather CORT relates to ecophysiological variation and individual energetics. This is particularly important to the use of feather CORT as a biomarker. Correlative work should

be substantiated with experiments that manipulate candidate energetic factors. Research at multiple levels of biological organization and spatial scales is needed to clarify some key points. We need a better understanding of the process by which CORT is deposited in feathers at the cellular and tissue levels. Does this process vary among individuals, life-history period, or species? Related to this, we need a better understanding of how feather tissue integrates baseline and stress-induced CORT levels. Does one explain more variation in feather CORT than the other? Field studies are essential to determining how frequently free-living birds actually encounter stressors. The idea that CORT in feathers is proportional to the severity of the challenge, as has been suggested for plasma CORT [15], should be tested experimentally. Additionally, it will be interesting to determine if different types of challenges (e.g., physical vs. psychological) differentially influence feather CORT. Studies should also address the sensitivity of feather CORT to variation in specific energetic demands within and among life-history periods. Identifying ecological factors and specific energetic demands that explain a disproportionate amount of variation in feather CORT will be particularly useful. Expanding the geographic scale of investigations using the CORT-energetics hypothesis may provide a physiological mechanism underlying species-energy relationships [317].

Although my research could not directly address the consequences of variation in feather CORT, it is interesting to note that using post-breeding feather CORT I was able connect, albeit correlatively, the consequences of one breeding season to behavior of the following breeding season (Chapter 5). As I suggest in Chapter 5, this begs the question of whether a carry-over effect was operating in tree swallows and, if so, if feather CORT quantified it. Although it is highly unlikely that CORT secreted during feather growth has

any direct influence on behaviour months later, CORT's role as a metabolic hormone could easily influence individual state (i.e., condition) following breeding and possibly into migration. Individual quality may influence how effects carry-over into subsequent periods [269], and my work suggests that feather CORT is related to quality (Chapter 5). Studies should investigate the potential relationship between feather CORT and carry-over effects.

Finally, additional research may improve the techniques by which we extract and measure CORT in feathers. The estimation of true extraction efficiency is hindered by the constraints of impregnating keratin tissue with radioactive tracer, and percent recovery of tracer from extract solution (i.e., filtering efficiency) is used as a proxy. A second extraction of feather material with methanol yielded no additional detectable CORT [105], suggesting that a single methanol extraction removes most, if not all, CORT from the feather tissue. The percent recovery of tracer from extract solution (i.e., recovery efficiency) is a meaningful metric in the extraction procedure as it as it provides a measure of reproducibility in this important step, but future studies should strive to develop a technique for estimating true extraction efficiency. It also remains a possibility that the RIA measures some compound(s) in addition to CORT [105]. Chromatography and mass spectroscopy work will be essential for identifying all potentially cross-reactive substances in feather extracts, and careful studies of antibody dynamics will determine which substance(s), if any, other than CORT is being detected by RIA.

I want to emphasize that these knowledge gaps need not invalidate studies using feather CORT. CORT from feathers relates significantly and meaningfully to numerous ecological factors, as I and others have shown. This strongly suggests that feather CORT provides relevant information about the physiological functioning of birds. Clarifying

mechanisms of hormone deposition in feathers, improving analytical techniques, and disentangling the ecological causes and consequences of feather CORT will only enrich our understanding of this important ecophysiological measure.

7.4.4. Conclusions

My thesis research provides a foundation upon which future work can refine our understanding of feather CORT. The application of feather CORT to addressing unresolved issues in avian CORT physiology is just beginning. It is clear, however, that feather CORT provides a physiological measure that is generally relevant to avian ecophysiology. My work and that of others suggests that approaching avian ecology studies from an energetics perspective may be beneficial because it will provide a common currency with which to understand individual responses to ecological variation as well as the influence of physiology on measures of performance and fitness. My work suggests that using this approach in the context of CORT may be useful for moving research towards a more holistic understanding of the role GCs play in mediating the relationship between birds and their environment and, ultimately, fitness. The energetics explanation is an attempt at providing a testable framework for such an approach and has wide applicability to studying theoretical and applied questions. The ecophysiological niche model is intended to provide a theoretical backdrop for understanding feather CORT values and how they may relate to fitness. It is my hope that by putting CORT into a context that both ecologists and physiologists can relate to, my ideas outlined above will make for more productive cross-discipline research.

LITERATURE CITED

1. West-Eberhard M. J. 1989 Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20, 249-278.

2. Wingfield J. C. 2003 Control of behavioural strategies for capricious environments. *Anim Behav* 66, 807-815.

3. Partridge L., Harvey P. H. 1988 The ecological context of life-history evolution. *Science* 241, 1449-1455.

4. Ketterson E. D., Nolan V. 1992 Hormones and life histories: an integrative approach. *Am Nat* 140, S33-S62.

5. Finch C. E., Rose M. R. 1995 Hormones and the physiological architecture of life-history evolution. *Q Rev Biol* 70, 1-52.

6. Nelson R. J. 2000 *An introduction to behavioral endocrinology*. Sunderland, Massachusetts: Sinauer Associates, Inc.

7. Dufty A. M., Clobert J., Moller A. P. 2002 Hormones, developmental plasticity and adaptation. *Trends Ecol Evol* 17, 190-196.

8. McEwen B. S., Wingfield J. C. 2003 The concept of allostasis in biology and biomedicine. *Horm Behav* 43, 2-15.

9. McEwen B. S. 2007 Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiol Rev* 87, 873-904.

10. Wingfield J. C., Kitaysky A. S. 2002 Endocrine responses to unpredictable environmental events: Stress or anti-stress hormones? *Integr Comp Biol* 42, 600-609.

11. Landys M. M., Ramenofsky M., Wingfield J. C. 2006 Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen Comp Endocrinol* 148, 132-149.

12. Selye H. 1946 The general adaptation syndrome and the diseases of adaptation. *J Clinical Endocrinol Metabol* 6, 117-230.

13. Sapolsky R. M., Romero L. M., Munck A. U. 2000 How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21, 55-89.

14. Dallman M. F., Strack A. M., Akana S. F., Bradbury M. J., Hanson E. S., Scribner K. A., Smith M. 1993 Feast and famine - critical role of glucocorticoids with insulin in daily energy-flow. *Front Neuroendocrinol* 14, 303-347.

15. Dallman M. F., Bhatnagar S. 2001 Chronic stress and energy balance: role of the hypothalamo-pituitary-adrenal axis. In: *Handbook of Physiology, Section 7: The Endocrine System, Vol. IV: Coping with the Environment: Neural and Endocrine Mechanisms* (McEwen B. S., editor). pp. 179-210. New York: Oxford University Press.

16. Strack A. M., Sebastian R. J., Schwartz M. W., Dallman M. F. 1995 Glucocorticoids and insulin - reciprocal signals for energy-balance. *Am J Physiol-Reg Integr Comp Physiol* 37, R142-R149.

17. Ricklefs R. E. 2001 *The Economy of Nature*. New York, NY: W. H. Freeman and Company.

18. Stearns S. C. 1989 Trade-offs in life-history evolution. *Funct Ecol* 3, 259-268.

19. Stearns S. C. 1992 *The Evolution of Life Histories*. New York: Oxford University Press.

20. Guyton A. C., Hall J. E. 2006 *Textbook of Medical Physiology*. Philadelphia, PA: Elsevier Saunders.

21. Carsia R. V., Harvey S. 2000 Adrenals. In: *Sturkie's Avian Physiology* (Whittow G. C., editor). pp. 489-537. London: Academic Press.

22. Breuner C. W., Wingfield J. C., Romero L. M. 1999 Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *J Exp Zool* 284, 334-342.

23. Silverin B., Wingfield J. C. 1998 Adrenocortical responses to stress in breeding pied flycatchers *Ficedula hypoleuca*: relation to latitude, sex and mating status. *J Avian Biol* 29, 228-234.

24. Wingfield J. C., Jacobs J., Hillgarth N. 1997 Ecological constraints and the evolution of hormone-behavior interrelationships. In: *Integrative Neurobiology of Affiliation* (Carter C. S., Lederhendler I. I., Kirkpatrick B., editors). pp. 22-41.

25. Romero L. M. 2002 Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen Comp Endocrinol* 128, 1-24.

26. Wingfield J. C., Kubokawa K., Ishida K., Ishii S., Wada M. 1995 The adrenocortical-response to stress in male bush warblers, *Cettia diphone* - a comparison of breeding populations in Honshu and Hokkaido, Japan. *Zool Sci* 12, 615-621.

27. Bokony V., Lendvai A. Z., Liker A., Angelier F., Wingfield J. C., Chastel O. 2009 Stress response and the value of reproduction: are birds prudent parents? *Am Nat* 173, 589-598.

28. Breuner C. W., Orchinik M., Hahn T. P., Meddle S. L., Moore I. T., Owen-Ashley N. T., Sperry T. S., Wingfield J. C. 2003 Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am J Physiol-Reg Integr Comp Physiol* 285, R594-R600.

29. Hau M., Ricklefs R. E., Wikelski M., Lee K. A., Brawn J. D. 2010 Corticosterone, testosterone and life-history strategies of birds. *Proc R Soc B* 277, 3203-3212.

30. Ricklefs R. E., Wikelski M. 2002 The physiology/life-history nexus. *Trends Ecol Evol* 17, 462-468.

31. Wingfield J. C., Maney D. L., Breuner C. W., Jacobs J. D., Lynn S., Ramenofsky M., Richardson R. D. 1998 Ecological bases of hormone-behavior interactions: The "emergency life history stage". *Am Zool* 38, 191-206.

32. Wingfield J. C., Moore M. C., Farner D. S. 1983 Endocrine responses to inclement weather in naturally breeding populations of white-crowned sparrows (*Zonotrichia leucophrys pugetensis*). *Auk* 100, 56-62.

33. Romero L. M., Reed J. M., Wingfield J. C. 2000 Effects of weather on corticosterone responses in wild free-living passerine birds. *Gen Comp Endocrinol* 118, 113-122.

34. Kitaysky A. S., Piatt J. F., Wingfield J. C., Romano M. 1999 The adrenocortical stress-response of black-legged kittiwake chicks in relation to dietary restrictions. *J Comp Physiol B Biochem Syst Environ Physiol* 169, 303-310.

35. Kitaysky A. S., Romano M. D., Piatt J. F., Wingfield J. C., Kikuchi M. 2005 The adrenocortical response of tufted puffin chicks to nutritional deficits. *Horm Behav* 47, 606-619.

36. Raouf S. A., Smith L. C., Brown M. B., Wingfield J. C., Brown C. R. 2006 Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. *Anim Behav* 71, 39-48.

37. Creel S. 2001 Social dominance and stress hormones. *Trends Ecol Evol* 16, 491-497.

38. Sapolsky R. M. 1993 Cortisol concentrations and the social significance of rank instability among wild baboons. *Psychoneuroendocrinology* 17, 701-709.

39. Scheuerlein A., Van't Hof T. J., Gwinner E. 2001 Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proc R Soc B* 268, 1575-1582.

40. Marra P. P., Holberton R. L. 1998 Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* 116, 284-292.

41. Suorsa P., Huhta E., Nikula A., Nikinmaa M., Jantti A., Helle H., Hakkarainen H. 2003 Forest management is associated with physiological stress in an old-growth forest passerine. *Proc R Soc B* 270, 963-969.

42. Cockrem J. F., Silverin B. 2002 Variation within and between birds in corticosterone responses of great tits (*Parus major*). *Gen Comp Endocrinol* 125, 197-206.

43. Cockrem J. F. 2007 Stress, corticosterone responses and avian personalities. *J Ornithol* 148, S169-S178.

44. Carere C., Groothuis T. G. G., Mostl E., Daan S., Koolhaas J. M. 2003 Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. *Horm Behav* 43, 540-548.

45. Evans M. R., Roberts M. L., Buchanan K. L., Goldsmith A. R. 2006 Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. *J Evol Biol* 19, 343-352.

46. Quillfeldt P., Masello J. F., Strange I. J., Buchanan K. L. 2006 Begging and provisioning of thin-billed prions, *Pachyptila belcheri*, are related to testosterone and corticosterone. *Anim Behav* 71, 1359-1369.

47. Kitaysky A. S., Wingfield J. C., Piatt J. F. 2001 Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behav Ecol* 12, 619-625.

48. Belthoff J. R., Dufty A. M. 1998 Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. *Anim Behav* 55, 405-415.

49. Silverin B. 1997 The stress response and autumn dispersal behaviour in willow tits. *Anim Behav* 53, 451-459.

50. Holberton R. L. 1999 Changes in patterns of corticosterone secretion concurrent with migratory fattening in a neotropical migratory bird. *Gen Comp Endocrinol* 116, 49-58.

51. Landys M. T. M., Wingfield J. C., Ramenofsky M. 2004 Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelli*. *Horm Behav* 46, 574-581.

52. Holberton R. L., Wilson C. M., Hunter M. J., Cash W. B., Sims C. G. 2007 The role of corticosterone in supporting migratory lipogenesis in the dark- eyed junco, *Junco hyemalis*: A model for central and peripheral regulation. *Physiol Biochem Zool* 80, 125-137.

53. Lupien S. J., McEwen B. S. 1997 The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Res Rev* 24, 1-27.

54. Pravosudov V. V. 2003 Long-term moderate elevation of corticosterone facilitates avian food-caching behaviour and enhances spatial memory. *Proc R Soc B* 270, 2599-2604.

55. Pravosudov V. V. 2005 Corticosterone and memory in birds. In: *Functional Avian Endocrinology* (Dawson A., Sharp P. J., editors). pp. 257-269. New Delhi, India: Narosa Publishing House.

56. Kitaysky A. S., Kitaiskaia E., Piatt J., Wingfield J. C. 2003 Benefits and costs of increased levels of corticosterone in seabird chicks. *Horm Behav* 43, 140-149.

57. Mettke-Hofmann C., Gwinner E. 2004 Differential assessment of environmental information in a migratory and a nonmigratory passerine. *Anim Behav* 68, 1079-1086.

58. Mettke-Hofmann C., Ebert C., Schmidt T., Steiger S., Stieb S. 2005 Personality traits in resident and migratory warbler species. *Behaviour* 142, 1357-1375.

59. Johnson E. O., Kamilaris T. C., Chrousos G. P., Gold P. W. 1992 Mechanisms of stress - a dynamic overview of hormonal and behavioral homeostasis. *Neurosci Biobehav Rev* 16, 115-130.

60. McEwen B. S., Biron C. A., Brunson K. W., Bulloch K., Chambers W. H., Dhabhar F. S., Goldfarb R. H., Kitson R. P., Miller A. H., Spencer R. L.*, et al* 1997 The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. *Brain Res Rev* 23, 79-133.

61. Rubolini D., Romano M., Boncoraglio G., Ferrari R. P., Martinelli R., Galeotti P., Fasola M., Saino N. 2005 Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Horm Behav* 47, 592-605.

62. Saino N., Suffritti C., Martinelli R., Rubolini D., Moller A. P. 2003 Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hirundo rustica*). *Behav Ecol* 14, 318-325.

63. Butler M. W., Leppert L. L., Dufty A. M. 2010 Effects of small increases in corticosterone levels on morphology, immune function, and feather development. *Physiol Biochem Zool* 83, 78-86.

64. Bortolotti G. R., Mougeot F., Martinez-Padilla J., Webster L. M. I., Piertney S. B. 2009 Physiological stress mediates the honesty of social signals. *PLoS ONE* 4, e4983.

65. Mougeot F., Martinez-Padilla J., Bortolotti G. R., Webster L. M. I., Piertney S. B. 2010 Physiological stress links parasites to carotenoid-based colour signals. *J Evol Biol* 23, 643-650.

66. McGraw K. J., Lee K., Lewin A. 2011 The effect of capture-and-handling stress on carotenoid-based beak coloration in zebra finches. *J Comp Physiol A-Neuroethol Sens Neural and Behav Physiol* 197, 683-691.

67. Wingfield J. C., Sapolsky R. M. 2003 Reproduction and resistance to stress: when and how. *J Neuroendocrinol* 15, 711-724.

68. Spee M., Marchal L., Lazin D., Le Maho Y., Chastel O., Beaulieu M., Raclot T. 2011 Exogenous corticosterone and nest abandonment: A study in a long-lived bird, the Adélie penguin. *Horm Behav* 60, 362-370.

69. Schmid B., Chastel O., Jenni L. 2011 The prolactin response to an acute stressor in relation to parental care and corticosterone in a short-lived bird, the Eurasian hoopoe *Gen Comp Endocrinol* 174, 22-29.

70. Bonier F., Martin P. R., Moore I. T., Wingfield J. C. 2009 Do baseline glucocorticoids predict fitness? *Trends Ecol Evol* 24, 634-642.

71. Bonier F., Moore I. T., Martin P. R., Robertson R. J. 2009 The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen Comp Endocrinol* 163, 208-213.

72. Done T., Gow E. A., Stutchbury B. J. M. 2011 Corticosterone stress response and plasma metabolite levels during breeding and molt in a free-living migratory songbird, the wood thrush (*Hylocichla mustelina*). *Gen Comp Endocrinol* 171, 176-182.

73. Henriksen R., Rettenbacher S., Groothuis T. G. G. 2011 Prenatal stress in birds: Pathways, effects, function and perspectives. *Neurosci Biobehav Rev* 35, 1484-1501.

74. Love O. P., Wynne-Edwards K. E., Bond L., Williams T. D. 2008 Determinants of within- and among-clutch variation in yolk corticosterone in the European starling. *Horm Behav* 53, 104-111.

75. Hayward L. S., Satterlee D. G., Wingfield J. C. 2005 Japanese quail selected for high plasma corticosterone response deposit high levels of corticosterone in their eggs. *Physiol Biochem Zool* 78, 1026-1031.

76. Hayward L. S., Wingfield J. C. 2004 Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *Gen Comp Endocrinol* 135, 365-371.

77. Love O. P., Chin E. H., Wynne-Edwards K. E., Williams T. D. 2005 Stress hormones: A link between maternal condition and sex-biased reproductive investment. *Am Nat* 166, 751-766.

78. Pryke S. R., Rollins L. A., Buttemer W. A., Griffith S. C. 2011 Maternal stress to partner quality is linked to adaptive offspring sex ratio adjustment. *Behav Ecol* 22, 717-722.

79. Chin E. H., Love O. P., Verspoor J. J., Williams T. D., Rowley K., Burness G. 2009 Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proc R Soc B* 276, 499-505.

80. Spencer K. A., Heidinger B. J., D'Alba L. B., Evans N. P., Monaghan P. 2010 Then versus now: effect of developmental and current environmental conditions on incubation effort in birds. *Behav Ecol* 21, 999-1004.

81. Spencer K. A., Verhulst S. 2007 Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*Taeniopygia guttata*). *Horm Behav* 51, 273-280.

82. Love O. P., Williams T. D. 2008 Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and post-natal developmental stress. *Horm Behav* 54, 496-505.

83. Spencer K. A., Evans N. P., Monaghan P. 2009 Postnatal stress in birds: a novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. *Endocrinology* 150, 1931-1934.

84. Blas J., Bortolotti G. R., Tella J. L., Baos R., Marchant T. A. 2007 Stress response during development predicts fitness in a wild, long lived vertebrate. *Proc Natl Acad Sci U S A* 104, 8880-8884.

85. Romero L. M., Wikelski M. 2001 Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. *Proc Natl Acad Sci U S A* 98, 7366-7370.

86. Romero L. M., Wikelski M. 2010 Stress physiology as a predictor of survival in Galapagos marine iguanas. *Proc R Soc B* 277, 3157-3162.

87. Breuner C. W., Patterson S. H., Hahn T. P. 2008 In search of relationships between the acute adrenocortical response and fitness. *Gen Comp Endocrinol* 157, 288-295.

88. Williams T. D. 2008 Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean'. *Philos T R Soc B* 363, 1687-1698.

89. Romero L. M. 2004 Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol* 19, 249-255.

90. Blanchard D. C., Sakai R. R., McEwen B., Weiss S. M., Blanchard R. J. 1993 Subordiation stress - behavioral, brain, and neuroendocrine correlates. *Behav Brain Res* 58, 113-121.

91. Ouyang J. Q., Hau M., Bonier F. 2011 Within seasons and among years: When are corticosterone levels repeatable? *Horm Behav* 60, 559-564.

92. Cockrem J. F., Barrett D. P., Candy E. J., Potter M. A. 2009 Corticosterone responses in birds: Individual variation and repeatability in Adélie penguins (*Pygoscelis adeliae*) and other species, and the use of power analysis to determine sample sizes. *Gen Comp Endocrinol* 163, 158-168.

93. Wasser S. K., Hunt K. E., Brown J. L., Cooper K., Crockett C. M., Bechert U., Millspaugh J. J., Larson S., Monfort S. L. 2000 A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *Gen Comp Endocrinol* 120, 260-275.

94. Mostl E., Palme R. 2002 Hormones as indicators of stress. *Domestic Anim Endocrinol* 23, 67-74.

95. Millspaugh J. J., Washburn B. E. 2004 Use of fecal glucocorticold metabolite measures in conservation biology research: considerations for application and interpretation. *Gen Comp Endocrinol* 138, 189-199.

96. Wasser S. K., Bevis K., King G., Hanson E. 1997 Noninvasive physiological measures of disturbance in the northern spotted owl. *Conserv Biol* 11, 1019-1022.

97. Cabezas S., Blas J., Marchant T. A., Moreno S. 2007 Physiological stress levels predict survival probabilities in wild rabbits. *Horm Behav* 51, 313-320.

98. Thiel D., Jenni-Eiermann S., Braunisch V., Palme R., Jenni L. 2008 Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: a new methodological approach. *J Appl Ecol* 45, 845-853.

99. Legagneux P., Gauthier G., Chastel O., Picard G., Bety J. 2011 Do glucocorticoids in droppings reflect baseline level in birds captured in the wild? A case study in snow geese. *Gen Comp Endocrinol* 172, 440-445.

100. Carlsson H. E., Lyberg K., Royo F., Hau J. 2007 Quantification of stress sensitive markers in single fecal samples do not accurately predict excretion of these in the pig. *Res Vet Sci* 82, 423-428.

101. Goymann W., Trappschuh M., Jensen W., Schwabl I. 2006 Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in European stonechats. *Horm Behav* 49, 644-653.

102. Raul J. S., Cirimele V., Ludes B., Kintz P. 2004 Detection of physiological concentrations of cortisol and cortisone in human hair. *Clinical Biochemistry* 37, 1105-1111.

103. Thieme D., Anielski P., Grosse J., Sachs H., Mueller R. K. 2003 Identification of anabolic steroids in serum, urine, sweat and hair - Comparison of metabolic patterns. *Analytica Chimica Acta* 483, 299-306.

104. Sheriff M. J., Dantzer B., Delehanty B., Palme R., Boonstra R. 2011 Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166, 869-887.

105. Bortolotti G. R., Marchant T. A., Blas J., German T. 2008 Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Funct Ecol* 22, 494-500.

106. Bortolotti G. R., Marchant T., Blas J., Cabezas S. 2009 Tracking stress: localisation, deposition and stability of corticosterone in feathers. *J Exp Biol* 212, 1477-1482.

107. Lattin C. R., Reed J. M., DesRochers D. W., Romero L. M. 2011 Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. *J Avian Biol* 42, 247-252.

108. Bortolotti G. R. 2010 Flaws and pitfalls in the chemical analysis of feathers: bad news-good news for avian chemoecology and toxicology. *Ecol Appl* 20, 1766-1774.

109. Breuner C. W., Orchinik M. 2002 Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175, 99-112.

110. Koren L., Mokady O., Karaskov T., Klein J., Koren G., Geffen E. 2002 A novel method using hair for determining hormonal levels in wildlife. *Anim Behav* 63, 403-406.

111. Liu D., Diorio J., Tannenbaum B., Caldji C., Francis D., Freedman A., Sharma S., Pearson D., Plotsky P. M., Meaney M. J. 1997 Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* 277, 1659-1662.

112. Lindstrom J. 1999 Early development and fitness in birds and mammals. *Trends Ecol Evol* 14, 343-348.

113. Müller C., Jenni-Eiermann S., Jenni L. 2009 Effects of a short period of elevated circulating corticosterone on postnatal growth in free-living Eurasian kestrels *Falco tinnunculus*. *J Exp Biol* 212, 1405-1412.

114. Wada H. 2008 Glucocorticoids: Mediators of vertebrate ontogenetic transitions. *Gen Comp Endocrinol* 156, 441-453.

115. Romero L. M., Reed J. M. 2005 Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp Biochem Phys A* 140, 73-79.

116. Sims C. G., Holberton R. L. 2000 Development of the corticosterone stress response in young northern mockingbirds (*Mimus polyglottos*). *Gen Comp Endocrinol* 119, 193-201.

117. Dawson W. R., Whittow G. C. 2000 Regulation of body temperature. In: *Sturkie's Avian Physiology* (Whittow G. C., editor. pp. 343-390. 5th ed. Toronto: Academic Press.

118. Visser G. H. 1998 Development of temperature regulation. In: *Avian growth and development: evolution within the altricial-precocial spectrum* (Starck J. M., Ricklefs R. E., editors). pp. 117-156. New York: Oxford University Press.

119. Dawson R. D., Lawrie C. C., O'Brien E. L. 2005 The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. *Oecologia* 144, 499-507.

120. Greño J. L., Belda E. J., Barba E. 2008 Influence of temperatures during the nestling period on post-fledging survival of great tit *Parus major* in a Mediterranean habitat. *J Avian Biol* 39, 41-49.

121. Martin T. E., Ghalambor C. K. 1999 Males feeding females during incubation. I. Required by microclimate or constrained by nest predation. *Am Nat* 153, 131-139.

122. Frigerio D., Dittami J., Mostl E., Kotrschal K. 2004 Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). *Gen Comp Endocrinol* 137, 29-36.

123. DuRant S. E., Romero L. M., Talent L. G., Hopkins W. A. 2008 Effect of exogenous corticosterone on respiration in a reptile. *Gen Comp Endocrinol* 156, 126-133.

124. Takahashi H., Iigo M., Ando K., Tachibana T., Denbow D. M., Furuse M. 2005 Regulation of body temperature by thyrotropin-releasing hormone in neonatal chicks. *Dev Brain Res* 157, 58-64.

125. Bize P., Stocker A., Jenni-Eiermann S., Gasparini J., Roulin A. 2010 Sudden weather deterioration but not brood size affects baseline corticosterone levels in nestling Alpine swifts. *Horm Behav* 58, 591-598.

126. Lobato E., Merino S., Moreno J., Morales J., Tomas G., la Puente J. M. D., Osorno J. L., Kuchar A., Mostl E. 2008 Corticosterone metabolites in blue tit and pied flycatcher droppings: Effects of brood size, ectoparasites and temperature. *Horm Behav* 53, 295-305.

127. Brown M. B., Brown C. R. 2009 Blood sampling reduces annual survival in cliff swallows (*Petrochelidon pyrrhonota*). *Auk* 126, 853-861.

128. Dufty A. M. 2008 Stress responsiveness in nestlings: A comparison of two sampling techniques. *Auk* 125, 225-229.

129. Coombs A. B., Bowman J., Garroway C. J. 2010 Thermal properties of tree cavities during winter in a northern hardwood forest. *J Wildl Manage* 74, 1875-1881.

130. Mertens J. A. L. 1977 Thermal conditions for successful breeding in great tits (*Parus major* L.) I. Relation to growth and development of temperature regulation in nestling great tits. *Oecologia* 28, 1-29.

131. Marsh R. L. 1980 Development of temperature regulation in nestling tree swallows. *Condor* 82, 461-463.

132. Harms N. J., Fairhurst G. D., Bortolotti G. R., Smits J. E. G. 2010 Variation in immune function, body condition, and feather corticosterone in nestling Tree Swallows (*Tachycineta bicolor*) on reclaimed wetlands in the Athabasca oil sands, Alberta, Canada. *Environ Pollut* 158, 841-848.

133. Fairhurst G. D., Frey M. D., Reichert J. F., Szelest I., Kelly D. M., Bortolotti G. R. 2011 Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. *PLoS ONE* 6, e17663.

134. Fairhurst G. D., Navarro J., González-Solís J., Marchant T. A., Bortolotti G. R. 2012 Feather corticosterone of a nestling seabird reveals consequences of sex-specific parental investment. *Proc R Soc B* 279, 177-184.

135. Perez J. H., Ardia D. R., Chad E. K., Clotfelter E. D. 2008 Experimental heating reveals nest temperature affects nestling condition in tree swallows (*Tachycineta bicolor*). *Biol Lett* 4, 468-471.

136. Webb D. R. 1993 Maternal-nestling contact geometry and heat transfer in an altricial bird. *J Therm Biol* 18, 117-124.

137. Webb D. R., King J. R. 1983 An analysis of the heat budgets of the eggs and nest of the white-crowned sparrow, *Zonotrichia leucophrys*, in relation to parental attentiveness. *Physiol Zool* 56, 493-505.

138. Klaassen M. 1994 Growth and energetics of tern chicks from temperate and polar environments. *Auk* 111, 525-544.

139. Pendlebury C. J., MacLeod M. G., Bryant D. M. 2004 Variation in temperature increases the cost of living in birds. *J Exp Biol* 207, 2065-2070.

140. Ricklefs R. E., Hainsworth F. R. 1968 Temperature regulation in nestling cactus wrens: development of homeothermy. *Condor* 70, 121-127.

141. Williams J. B. 1988 Field metabolism of tree swallows during the breeding season. *Auk* 105, 706-714.

142. Romero L. M., Dickens M. J., Cyr N. E. 2009 The reactive scope model - A new model integrating homeostasis, allostasis, and stress. *Horm Behav* 55, 375-389.

143. Lombardo M. P., Bosman R. M., Faro C. A., Houtteman S. G., Kluisza T. S. 1995 Effect of feathers as nest insulation on incubation behavior and reproductive performance of Tree Swallows (*Tachycineta bicolor*). *Auk* 112, 973-981.

144. Stephenson S., Hannon S., Proctor H. 2009 The function of feathers in tree swallow nests: insulation or ectoparasite barrier? *Condor* 111, 479-487.

145. Blas J., Baos R., Bortolotti G. R., Marchant T. A., Hiraldo F. 2006 Age-related variation in the adrenocortical response to stress in nestling white storks (*Ciconia ciconia*) supports the developmental hypothesis. *Gen Comp Endocrinol* 148, 172-180.

146. Wada H., Cristol D. A., McNabb F. M. A., Hopkins W. A. 2009 Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. *Environ Sci Technol* 43, 6031-6038.

147. Stevenson I. R., Bryant D. M. 2000 Avian phenology - Climate change and constraints on breeding. *Nature* 406, 366-367.

148. Williams G. C. 1966 Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Am Nat* 100, 687-690.

149. Erikstad K. E., Fauchald P., Tveraa T., Steen H. 1998 On the cost of reproduction in long-lived birds: The influence of environmental variability. *Ecology* 79, 1781-1788.

150. Mauck R. A., Grubb T. C. 1995 Petrel parents shunt all experimentally increased reproductive costs to their offspring. *Anim Behav* 49, 999-1008.

151. Weimerskirch H., Chastel O., Ackermann L. 1995 Adjustment of parental effort to manipulated foraging ability in a pelagic seabird, the thin-billed prion *Pachyptila belcheri*. *Behav Ecol Sociobiol* 36, 11-16.

152. Weimerskirch H., Fradet G., Cherel Y. 1999 Natural and experimental changes in chick provisioning in a long-lived seabird, the Antarctic prion. *J Avian Biol* 30, 165-174.

153. Navarro J., González-Solís J. 2007 Experimental increase of flying costs in a pelagic seabird: effects on foraging strategies, nutritional state and chick condition. *Oecologia* 151, 150-160.

154. Velando A., Alonso-Alvarez C. 2003 Differential body condition regulation by males and females in response to experimental manipulations of brood size and parental effort in the blue-footed booby. *J Anim Ecol* 72, 846-856.

155. González-Solís J., Croxall J. P., Wood A. G. 2000 Sexual dimorphism and sexual segregation in foraging strategies of northern giant petrels, *Macronectes halli*, during incubation. *Oikos* 90, 390-398.

156. González-Solís J., Croxall J. P., Wood A. G. 2000 Foraging partitioning between giant petrels *Macronectes* spp. and its relationship with breeding population changes at Bird Island, South Georgia. *Mar Ecol Prog Ser* 204, 279-288.

157. Lewis S., Benvenuti S., Dall'Antonia L., Griffiths R., Money L., Sherratt T. N., Wanless S., Hamer K. C. 2002 Sex-specific foraging behaviour in a monomorphic seabird. *Proc R Soc B* 269, 1687-1693.

158. Quillfeldt P., Masello J. F., Hamer K. C. 2004 Sex differences in provisioning rules and honest signalling of need in Manx shearwaters, *Puffinus puffinus*. *Anim Behav* 68, 613-620.

159. Weimerskirch H., Cherel Y., CuenotChaillet F., Ridoux V. 1997 Alternative foraging strategies and resource allocation by male and female Wandering Albatrosses. *Ecology* 78, 2051-2063.

160. Gray C. M., Hamer K. C. 2001 Food-provisioning behaviour of male and female Manx shearwaters, *Puffinus puffinus*. *Anim Behav* 62, 117-121.

161. Hamer K. C., Quillfeldt P., Masello J. F., Fletcher K. L. 2006 Sex differences in provisioning rules: responses of Manx shearwaters to supplementary chick feeding. *Behav Ecol* 17, 132-137.

162. Peck D. R., Congdon B. C. 2006 Sex-specific chick provisioning and diving behaviour in the wedge-tailed shearwater *Puffinus pacificus*. *J Avian Biol* 37, 245-251.

163. Elliott K. H., Gaston A. J., Crump D. 2010 Sex-specific behavior by a monomorphic seabird represents risk partitioning. *Behav Ecol* 21, 1024-1032.

164. Kitaysky A. S., Kitaiskaia E. V., Wingfield J. C., Piatt J. F. 2001 Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *J Comp Physiol B Biochem Syst Environ Physiol* 171, 701-709.

165. Kitaysky A. S., Kitaiskaia E. V., Piatt J. F., Wingfield J. C. 2006 A mechanistic link between chick diet and decline in seabirds? *Proc R Soc B* 273, 445-450.

166. Harding A. M. A., Kitaysky A. S., Hall M. E., Welcker J., Karnovsky N. J., Talbot S. L., Hamer K. C., Gremillet D. 2009 Flexibility in the parental effort of an Arctic-breeding seabird. *Funct Ecol* 23, 348-358.

167. Kitaysky A. S. 1999 Metabolic and developmental responses of alcid chicks to experimental variation in food intake. *Physiol Biochem Zool* 72, 462-473.

168. Apanius V. 1998 Stress and immune defense. In: *Stress and Behavior* (Møller A. P., Milinski M., Slater P. J. B., editors). pp. 133-153. London: Academic Press.

169. Sears J., Hatch S. A. 2008 Rhinoceros auklet developmental responses to food limitation: an experimental study. *Condor* 110, 709-717.

170. Navarro J., Forero M. G., González-Solís J., Igual J. M., Becares J., Hobson K. A. 2009 Foraging segregation between two closely related shearwaters breeding in sympatry. *Biol Lett* 5, 545-548.

171. Granadeiro J. P., Nunes M., Silva M. C., Furness R. W. 1998 Flexible foraging strategy of Cory's shearwater, *Calonectris diomedea*, during the chick-rearing period. *Anim Behav* 56, 1169-1176.

172. Weimerskirch H., Ancel A., Caloin M., Zahariev A., Spagiari J., Kersten M., Chastel O. 2003 Foraging efficiency and adjustment of energy expenditure in a pelagic seabird provisioning its chick. *J Anim Ecol* 72, 500-508.

173. Zino P. A., Zino F., Maul T., Biscoito J. M. 1987 The laying, incubation and fledging periods of Cory's shearwater *Calonectris diomedea borealis* on Selvagem Grande in 1984. *Ibis* 129, 393-398.

174. Warham J. 1990 *The Petrels. Their Ecology and Breeding Systems*. London: Academic Press.

175. Pennycuick C. J. 1989 Variables needed for flight calculations. In: *Bird flight performance* (Pennycuick C. J., editor). pp. 7-17. Oxford: Oxford University Press.

176. Passos C., Navarro J., Giudici A., González-Solís J. 2010 Effects of extra mass on the pelagic behavior of a seabird. *Auk* 127, 100-107.

177. Wayland M., Gilchrist H. G., Marchant T., Keating J., Smits J. E. 2002 Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. *Environ Res* 90, 47-60.

178. Ottosson U., Backman J., Smith H. G. 1997 Begging affects parental effort in the pied flycatcher, *Ficedula hypoleuca*. *Behav Ecol Sociobiol* 41, 381-384.

179. Weimerskirch H., Mougey T., Hindermeyer X. 1997 Foraging and provisioning strategies of black-browed albatrosses in relation to the requirements of the chick: natural variation and experimental study. *Behav Ecol* 8, 635-643.

180. Gladbach A., Braun C., Nordt A., Peter H. U., Quillfeldt P. 2009 Chick provisioning and nest attendance of male and female Wilson's storm petrels *Oceanites oceanicus*. *Polar Biol* 32, 1315-1321.

181. Smith S. B., McWilliams S. R. 2009 Dietary macronutrients affect lipid metabolites and body composition of a migratory passerine, the white-throated sparrow (*Zonotrichia albicollis*). *Physiol Biochem Zool* 82, 258-269.

182. Spencer K. A., Buchanan K. L., Goldsmith A. R., Catchpole C. K. 2003 Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Horm Behav* 44, 132-139.

183. Sockman K. W., Schwabl H. 2001 Plasma corticosterone in nestling American kestrels: Effects of age, handling stress, yolk androgens, and body condition. *Gen Comp Endocrinol* 122, 205-212.

184. Adams N. J., Cockrem J. F., Candy E. J., Taylor G. A. 2008 Non-precocial grey-faced petrel chicks (*Pterodroma macroptera gouldi*) show no age-related variation in corticosterone responses to capture and handling. *Gen Comp Endocrinol* 157, 86-90.

185. Jones R. B. 1996 Fear and adaptability in poultry: Insights, implications and imperatives. *Worlds Poultry Science Journal* 52, 131-174.

186. Newberry R. C. 1995 Environmental enrichment: increasing the biological relevance of captive environments. *Appl Anim Behav Sci* 44, 229-243.

187. de Azevedo C. S., Cipreste C. F., Young R. J. 2007 Environmental enrichment: A GAP analysis. *Appl Anim Behav Sci* 102, 329-343.

188. Morgan K. N., Tromborg C. T. 2007 Sources of stress in captivity. *Appl Anim Behav Sci* 102, 262-302.

189. Leone E. H., Estevez I. 2008 Economic and welfare benefits of environmental enrichment for broiler breeders. *Poult Sci* 87, 14-21.

190. Mason G., Clubb R., Latham N., Vickery S. 2007 Why and how should we use environmental enrichment to tackle stereotypic behaviour? *Appl Anim Behav Sci* 102, 163-188.

191. Mendl M., Burman O. H. P., Parker R. M. A., Paul E. S. 2009 Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Appl Anim Behav Sci* 118, 161-181.

192. Mettke-Hofmann C. 2007 Object exploration of garden and sardinian warblers peaks in spring. *Ethology* 113, 174-182.

193. Sabbatini G., Stammati M., Tavares M. C. H., Visalberghi E. 2007 Response toward novel stimuli in a group of tufted capuchins (*Cebus libidinosus*) in Brasilia National Park, Brazil. *Am J Primatol* 69, 457-470.

194. Mettke-Hofmann C., Wink M., Winkler H., Leisler B. 2005 Exploration of environmental changes relates to lifestyle. *Behav Ecol* 16, 247-254.

195. Fox R. A., Ladage L. D., Roth T. C., Pravosudov V. V. 2009 Behavioural profile predicts dominance status in mountain chickadees, *Poecile gambeli*. *Anim Behav* 77, 1441-1448.

196. McDougall P. T., Reale D., Sol D., Reader S. M. 2006 Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Anim Conserv* 9, 39-48.

197. Fox R. A., Millam J. R. 2007 Novelty and individual differences influence neophobia in orange-winged Amazon parrots (*Amazona amazonica*). *Appl Anim Behav Sci* 104, 107-115.

198. Balcombe J. P. 2006 Laboratory environments and rodents' behavioural needs: a review. *Laboratory Animals* 40, 217-235.

199. Lutz C. K., Novak M. A. 2005 Environmental enrichment for nonhuman primates: Theory and application. *ILAR J* 46, 178-191.

200. Meehan C. L., Mench J. A. 2002 Environmental enrichment affects the fear and exploratory responses to novelty of young Amazon parrots. *Appl Anim Behav Sci* 79, 75-88.

201. deMonte M., LePape G. 1997 Behavioural effects of cage enrichment in single-caged adult cats. *Anim Welf* 6, 53-66.

202. Kells A., Dawkins M. S., Borja M. C. 2001 The effect of a 'freedom food' enrichment on the behaviour of broilers on commercial farms. *Anim Welf* 10, 347-356.

203. Cockrem J. F., Silverin B. 2002 Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). *Gen Comp Endocrinol* 125, 248-255.

204. Mettke-Hofmann C., Rowe K. C., Hayden T. J., Canoine V. 2006 Effects of experience and object complexity on exploration in garden warblers (*Sylvia borin*). *J Zool* 268, 405-413.

205. Cummings D., Brown J. L., Rodden M. D., Songsasen N. 2007 Behavioral and physiologic responses to environmental enrichment in the maned wolf (*Chrysocyon brachyurus*). *Zoo Biol* 26, 331-343.

206. Wingfield J. C., Ramenofsky M. 1999 Hormones and the behavioral ecology of stress. In: *Stress Physiology in Animals* (Balm P. H. M., editor). pp. 1-51. Sheffield: Academic Press.

207. Dobson H., Smith R. F. 1995 Stress and reproduction in farm animals. *J Reprod Fertil*, 451-461.

208. Charmandari E. 2005 Endocrinology of the stress response. *Annu Rev Physiol* 67, 259-284.

209. Boinski S., Swing S. P., Gross T. S., Davis J. K. 1999 Environmental enrichment of brown capuchins (*Cebus apella*): Behavioral and plasma and fecal cortisol measures of effectiveness. *Am J Primatol* 48, 49-68.

210. Pohle K., Cheng H. W. 2009 Comparative effects of furnished and battery cages on egg production and physiological parameters in white leghorn hens. *Poult Sci* 88, 2042-2051.

211. Barnett J. L., Tauson R., Downing J. A., Janardhana V., Lowenthal J. W., Butler K. L., Cronin G. M. 2009 The effects of a perch, dust bath, and nest box, either alone or in combination as used in furnished cages, on the welfare of laying hens. *Poult Sci* 88, 456-470.

212. van de Weerd H. A., Day J. E. L. 2009 A review of environmental enrichment for pigs housed in intensive housing systems. *Appl Anim Behav Sci* 116, 1-20.

213. Marashi V., Barnekow A., Ossendorf E., Sachser N. 2003 Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Horm Behav* 43, 281-292.

214. Moncek F., Duncko R., Johansson B. B., Jezova D. 2004 Effect of environmental enrichment on stress related systems in rats. *J Neuroendocrinol* 16, 423-431.

215. Fox C., Merali Z., Harrison C. 2006 Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. *Behav Brain Res* 175, 1-8.

216. Pena Y., Prunell M., Rotllant D., Armario A., Escorihuela R. M. 2009 Enduring effects of environmental enrichment from weaning to adulthood on pituitary-adrenal function, pre-pulse inhibition and learning in male and female rats. *Psychoneuroendocrinology* 34, 1390-1404.

217. Ishiwata T., Uetake K., Abe N., Eguchi Y., Tanaka T. 2006 Effects of an environmental enrichment using a drum can on behavioral, physiological and productive characteristics in fattening beef cattle. *Anim Sci J* 77, 352-362.

218. Veissier I., Chazal P., Pradel P., LeNeindre P. 1997 Providing social contacts and objects for nibbling moderates reactivity and oral behaviors in veal calves. *J Anim Sci* 75, 356-365.

219. Dybkjaer L. 1992 The identification of behavioural indicators of stress in early weaned piglets. *Appl Anim Behav Sci* 35, 135-147.

220. Gardner J. M., Duncan I. J. H., Widowski T. M. 2001 Effects of social "stressors" on belly-nosing behaviour in early-weaned piglets: is belly-nosing an indicator of stress? *Appl Anim Behav Sci* 74, 135-152.

221. Le Maho Y., Karmann H., Briot D., Handrich Y., Robin J. P., Mioskowski E., Cherel Y., Farni J. 1992 Stress in birds due to routine handling and a technique to avoid it. *Am J Physiol* 263, R775-R781.

222. Bolhuis J. E., Schouten W. G. P., Schrama J. W., Wiegant V. M. 2005 Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. *Appl Anim Behav Sci* 93, 213-228.

223. Pohle K., Cheng H. W. 2009 Furnished cage system and hen well-being: Comparative effects of furnished cages and battery cages on behavioral exhibitions in White Leghorn chickens. *Poult Sci* 88, 1559-1564.

224. Bateson M., Matheson S. M. 2007 Performance on a categorisation task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). *Anim Welf* 16, 33-36.

225. Rushen J. 2000 Some issues in the interpretation of behavioural responses to stress. In: *The Biology of Animal Stress - Basic principles and implications for animal welfare* (Moberg G. P., Mench J. A., editors). pp. 23-42. New York: CAGI Publishing.

226. Paul E. S., Harding E. J., Mendl M. 2005 Measuring emotional processes in animals: the utility of a cognitive approach. *Neurosci Biobehav Rev* 29, 469-491.

227. Young R. J. 2003 *Environmental enrichment for captive animals*. Oxford: Blackwell Science.

228. Carlstead K., Shepherdson D. 2000 Alleviating stress in zoo animals with environmental enrichment. In: *The Biology of Animal Stress - Basic principles and implications for animal welfare* (Moberg G. P., Mench J. A., editors). pp. 337-354. New York: CABI Publishing.

229. Wolfe T. L. 2000 Understanding the role of stress in animal welfare: practical considerations In: *The Biology of Animal Stress - Basic principles and implications for animal welfare* (Moberg G. P., Mench J. A., editors). pp. 355-368. New York: CABI Publishing.

230. Hemsworth P. H. 2003 Human-animal interactions in livestock production. *Appl Anim Behav Sci* 81, 185-198.

231. Boissy A., Manteuffel G., Jensen M. B., Moe R. O., Spruijt B., Keeling L. J., Winckler C., Forkman B., Dimitrov I., Langbein J.*, et al* 2007 Assessment of positive emotions in animals to improve their welfare. *Physiol Behav* 92, 375-397.

232. Wasser S. K., Hunt K. E. 2005 Noninvasive measures of reproductive function and disturbance in the barred owl, great horned owl, and northern spotted owl. In: *Bird Hormones and Bird Migrations: Analyzing Hormones in Droppings and Egg Yolks and Assessing Adaptations in Long-Distance Migration* (Goymann W., Jenni-Eiermann S., Bauchinger U., editors). pp. 109-137. New York: Wiley-Blackwell.

233. Staley A. M., Blanco J. M., Dufty A. M., Wildt D. E., Monfort S. L. 2007 Fecal steroid monitoring for assessing gonadal and adrenal activity in the golden eagle and peregrine falcon. *J Comp Physiol B Biochem Syst Environ Physiol* 177, 609-622.

234. Menargues A., Urios V., Mauri M. 2008 Welfare assessment of captive Asian elephants (*Elephas maximus*) and Indian rhinoceros (*Rhinoceros unicornis*) using salivary cortisol measurement. *Anim Welf* 17, 305-312.

235. Tomback D. F. 1998 Clark's nutcracker. *Birds N Am* 331, 1-24.

236. Greenberg R., Mettke-Hofmann C. 2001 Ecological aspects of neophobia and neophilia in birds. In: *Current Ornithology* (Nolan V., Jr., Thompson C. F., editors). pp. 119-178. New York: Kluwer Academic/Plenum.

237. Mettke-Hofmann C., Winkler H., Leisler B. 2002 The significance of ecological factors for exploration and neophobia in parrots. *Ethology* 108, 249-272.

238. Fox R. A., Millam J. R. 2004 The effect of early environment on neophobia in orange-winged Amazon parrots (*Amazona amazonica*). *Appl Anim Behav Sci* 89, 117-129.

239. Littell R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, O. Schabenberger 2006 *SAS for Mixed Models, Second Edition*. Cary, NC: SAS Institute, Inc.

240. Dobrakovova M., Kvetnansky R., Oprsalova Z., Jezova D. 1993 Specificity of the effect of repeated handling on sympathetic-adrenomedullary and pituitary-adrenocortical activity in rats. *Psychoneuroendocrinology* 18, 163-174.

241. Bosson C. O., Palme R., Boonstra R. 2009 Assessment of the stress response in columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. *Physiol Biochem Zool* 82, 291-301.

242. Cornelius J. M., Breuner C. W., Hahn T. P. 2010 Under a neighbour's influence: public information affects stress hormones and behaviour of a songbird. *Proc R Soc B* 277, 2399-2404.

243. Gagliano H., Fuentes S., Nadal R., Armario A. 2008 Previous exposure to immobilisation and repeated exposure to a novel environment demonstrate a marked dissociation between behavioral and pituitary-adrenal responses. *Behav Brain Res* 187, 239-245.

244. Renner M. J., Rosenzweig M. R. 1987 *Enriched and Impoverished Environments - Effects on Brain and Behavior*. New York: Springer-Verlag.

245. Morley-Fletcher S., Rea M., Maccari S., Laviola G. 2003 Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *Eur J Neurosci* 18, 3367-3374.

246. Gill F. B. 2007 *Ornithology*. New York: W. H. Freeman and Company.

247. Carey C. 1996 Female reproductive energetics. In: *Avian energetics and nutritional ecology* (Carey C., editor). pp. 324-374. Toronto: Chapman and Hall.

248. Williams J. B. 1996 Energetics of avian incubation. In: *Avian energetics and nutritional ecology* (Carey C., editor). pp. 375-415. Toronto: Chapman and Hall.

249. Ilmonen P., Taarna T., Hasselquist D. 2000 Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proc R Soc B* 267, 665-670.

250. Love O. P., Breuner C. W., Vezina F., Williams T. D. 2004 Mediation of a corticosterone-induced reproductive conflict. *Horm Behav* 46, 59-65.

251. Bonier F., Moore I. T., Robertson R. J. 2011 The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. *Biol Lett* 7, 944-966.

252. Ouyang J. Q., Sharp P. J., Dawson A., Quetting M., Hau M. 2011 Hormone levels predict individual differences in reproductive success in a passerine bird *Proc R Soc B* 278, 2537-2545.

253. Patterson S. H., Winkler D. W., Breuner C. W. 2011 Glucocorticoids, individual quality and reproductive investment in a passerine bird. *Anim Behav* 81, 1239-1247.

254. Verhulst S., Nilsson J. A. 2008 The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philos T R Soc B* 363, 399-410.

255. Smith R. J., Moore F. R. 2005 Arrival timing and seasonal reproductive performance in a long-distance migratory landbird. *Behav Ecol Sociobiol* 57, 231-239.

256. Nilsson S. G. 1984 The evolution of nest-site selection among hole-nesting birds: the importance of nest predation and competition. *Ornis Scand* 15, 167-175.

257. Verhulst S., Tinbergen J. M. 1991 Experimental evidence for a causal relationship between timing and success of reproduction in a the great tit *Parus m. major*. *J Anim Ecol* 60, 269-282.

258. Garcia-Navas V., Sanz J. J. 2011 Seasonal decline in provisioning effort and nestling mass of blue tits *Cyanistes caeruleus*: experimental support for the parent quality hypothesis. *Ibis* 153, 59-69.

259. Rhodes B., O'Donnell C., Jamieson I. 2009 Microclimate of natural cavity nests and its implications for a threatened secondary-cavity-nesting passerine of New Zealand, the South Island Saddleback. *Condor* 111, 462-469.

260. Murphy M. T., Armbrecth B., Vlamis E., Pierce A. 2000 Is reproduction by tree swallows cost free? *Auk* 117, 902-912.

261. Winkler D. W., Allen P. E. 1996 The seasonal decline in tree swallow clutch size: Physiological constraint or strategic adjustment? *Ecology* 77, 922-932.

262. Quinn G. P., Keough M. J. 2002 *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.

263. Leffelaar D., Robertson R. J. 1986 Equality of feeding roles and the maintenance of monogamy in tree swallows. *Behav Ecol Sociobiol* 18, 199-206.

264. Burness G. P., Ydenberg R. C., Hochachka P. W. 2001 Physiological and biochemical correlates of brood size and energy expenditure in tree swallows. *J Exp Biol* 204, 1491-1501.

265. Lobato E., Moreno J., Merino S., Morales J., Tomas G., Martinez J., Vasquez R. A., Kuchar A., Mostl E., Osorno J. L. 2010 Arrival date and territorial behavior are associated with corticosterone metabolite levels in a migratory bird. *J Ornithol* 151, 587-597.

266. Forero M. G., Donazar J. A., Blas J., Hiraldo F. 1999 Causes and consequences of territory change and breeding dispersal distance in the black kite. *Ecology* 80, 1298-1310.

267. Dingemanse N. J., Reale D. 2005 Natural selection and animal personality. *Behaviour* 142, 1159-1184.

268. Dingemanse N. J., Both C., Drent P. J., Tinbergen J. M. 2004 Fitness consequences of avian personalities in a fluctuating environment. *Proc R Soc B* 271, 847-852.

269. Harrison X. A., Blount J. D., Inger R., Norris D. R., Bearhop S. 2011 Carry-over effects as drivers of fitness differences in animals. *J Anim Ecol* 80, 4-18.

270. Saunders D. A., Hobbs R. J., Margules C. R. 1991 Biological consequences of ecosystem fragmentation: a review. *Conserv Biol* 5, 18-32.

271. Andrén H. 1994 Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* 71, 355-366.

272. Wikelski M., Cooke S. J. 2006 Conservation physiology. *Trends Ecol Evol* 21, 38-46.

273. Busch D. S., Hayward L. S. 2009 Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biol Conserv* 142, 2844-2853.

274. Wingfield J. C., Vleck C. M., Moore M. C. 1992 Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. *J Exp Zool* 264, 419-428.

275. Homyack J. A. 2010 Evaluating habitat quality of vertebrates using conservation physiology tools. *Wildl Res* 37, 332-342.

276. Hobson K. A., Wassenaar L. I., editors (2008) Tracking animal migration with stable isotopes. New York: Academic Press.

277. Inger R., Bearhop S. 2008 Applications of stable isotope analyses to avian ecology. *Ibis* 150, 447-461.

278. Kelly J. F. 2000 Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can J Zool* 78, 1-27.

279. Hobson K. A. 1999 Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: Implications for evaluating trophic relationships and breeding origins. *Condor* 101, 799-805.

280. Vögeli M., Serrano D., Pacios F., Tella J. L. 2010 The relative importance of patch habitat quality and landscape attributes on a declining steppe-bird metapopulation. *Biol Conserv* 143, 1057-1067.

281. Mazerolle D. F., Hobson K. A. 2002 Physiological ramifications of habitat selection in territorial male ovenbirds: consequences of landscape fragmentation. *Oecologia* 130, 356-363.

282. Kilgas P., Mand R., Magi M., Tilgar V. 2006 Hematological parameters in brood-rearing great tits in relation to habitat, multiple breeding and sex. *Comp Biochem Phys A* 144, 224-231.

283. Instituto Aragonés de Estadistica (2011) Clima. http://www.aragon.es/DepartamentosOrganismosPublicos/Organismos/InstitutoAragonesEstadistica.

284. Gobierno de Navarra (2011) Meteorología y climatología de Navarra. http://meteo.navarra.es/.

285. Cramp S., editor (1988) Handbook of the Birds of Europe, the Middle East and North Africa. Oxford: Oxford University Press.

286. Herranz J., Yanes M., Suárez F. 1993 First record on the diet of Dupont's lark *Chersophilus duponti* nestlings in the Iberian Peninsula. *Ardeola* 40, 77-79.

287. Laiolo P. 2008 Characterizing the spatial structure of songbird cultures. *Ecol Appl* 18, 1774-1780.

288. Laiolo P., Vögeli M., Serrano D., Tella J. L. 2008 Song diversity predicts the viability of fragmented bird populations. *PLoS ONE* 3.

289. Vögeli M., Laiolo P., Serrano D., Tella J. L. 2008 Who are we sampling? Apparent survival differs between methods in a secretive species. *Oikos* 117, 1816-1823.

290. Vögeli M., Serrano D., Tella J. L., Méndez M., Godoy J. A. 2007 Sex determination Duponts lark *Chersophilus duponti* using molecular sexing and discriminant functions. *Ardeola* 54, 69-79.

291. Svensson L. 1992 *Identification Guide to European Passerines*. Stockholm: British Trust for Ornithology.

292. SIGPAC (2011) Sistema de información geográfica de parcelas agrícolas (SIGPAC). http://www.marm.es/es/cartografia-y-sig/temas/sistema-de-informacion-geografica-de-parcelas-agricolas-sigpac-/default.aspx.

293. Seoane J., Justribo J. H., Garcia F., Retamar J., Rabadan C., Atienza J. C. 2006 Habitat-suitability modelling to assess the effects of land-use changes on Dupont's lark *Chersophilus duponti*: A case study in the Layna Important Bird Area. *Biol Conserv* 128, 241-252.

294. ESRI (2004) ArcGIS 9. Redlands, CA: ESRI, Inc.

295. Braun-Blanquet J., de Bolós O. 1957 Les groupement végétaux du bassin moyen de l'Ebre et leur dynamisme. *Anales de la Estación Experimental de Aula Dei* 5, 1-266.

296. Hanski I., Kuussaari M., Nieminen M. 1994 Metapopulation structure and migration in the butterly *Melitaea cinxia*. *Ecology* 75, 747-762.

297. Coplen T. B., Brand W. A., Gehre M., Groning M., Meijer H. A. J., Toman B., Verkouteren R. M. 2006 New guidelines for $\delta^{13}C$ measurements. *Analytical Chemistry* 78, 2439-2441.

298. Sokal R. R., Oden N. L. 1978 Spatial autocorrelation in biology. 1. Methodology. *Biol J Linn Soc* 10, 199-228.

299. Legendre P., Legendre L. 1998 *Numerical Ecology*. Amsterdam: Elsevier.

300. Rangel T. F., Diniz J. A. F., Bini L. M. 2010 SAM: a comprehensive application for Spatial Analysis in Macroecology. *Ecography* 33, 46-50.

301. Burnham K. P., Anderson D. R. 2002 *Model selection and multiple inference: a practical information-theoretic approach*. Heidlberg: Springer.

302. Bolker B. M., Brooks M. E., Clark C. J., Geange S. W., Poulsen J. R., Stevens M. H. H., White J. S. S. 2009 Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24, 127-135.

303. Hobson K. A., Bairlein F. 2003 Isotopic fractionation and turnover in captive garden warblers (*Sylvia borin*): implications for delineating dietary and migratory associations in wild passerines. *Can J Zool* 81, 1630-1635.

304. Podlesak D. W., McWilliams S. R. 2006 Metabolic routing of dietary nutrients in birds: Effects of diet quality and macronutrient composition revealed using stable isotopes. *Physiol Biochem Zool* 79, 534-549.

305. Diggs N. E., Marra P. P., Cooper R. J. 2011 Resource limitation drives patterns of habitat occupancy during the nonbreeding season for an omnivorous songbird. *Condor* 113, 646-654.

306. Sahin K., Sahin N., Onderci M., Gursu F., Cikim G. 2002 Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. *Biol Trace Elem Res* 89, 53-64.

307. Polis G. A., Anderson W. B., Holt R. D. 1997 Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. *Annu Rev Ecol Syst* 28, 289-316.

308. Vögeli M., Lemus J. A., Serrano D., Blanco G., Tella J. L. 2011 An island paradigm at mainland: population fragmentation impairs the community of avian pathogens. *Proc R Soc B* 278, 2668-2676.

309. Méndez M., Tella J. L., Godoy J. A. 2011 Restricted gene flow and genetic drift in recently fragmented populations of an endangered steppe bird. *Biol Conserv* 144, 2615-2622.

310. Fokidis H. B., Orchinik M., Deviche P. 2011 Context-specific territorial behavior in urban birds: No evidence for involvement of testosterone or corticosterone. *Horm Behav* 59, 133-143.

311. Cyr N. E., Wikelski M., Romero L. M. 2008 Increased energy expenditure but decreased stress responsiveness during molt. *Physiol Biochem Zool* 81, 452-462.

312. Holberton R. L., Able K. P. 2000 Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). *Proc R Soc B* 267, 1889-1896.

313. Heidinger B. J., Nisbet I. C. T., Ketterson E. D. 2006 Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? *Proc R Soc B* 273, 2227-2231.

314. James F. C. 1970 Geographic size variation in birds and its relationship to climate. *Ecology* 51, 365-390.

315. Wikelski M., Spinney L., Schelsky W., Scheuerlein A., Gwinner E. 2003 Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. *Proc R Soc B* 270, 2383-2388.

316. Piersma T., Lindstrom A., Drent R. H., Tulp I., Jukema J., Morrison R. I. G., Reneerkens J., Schekkerman H., Visser G. H. 2003 High daily energy expenditure of incubating shorebirds on High Arctic tundra: a circumpolar study. *Funct Ecol* 17, 356-362.

317. Evans K. L., Greenwood J. J. D., Gaston K. J. 2005 Dissecting the species-energy relationship. *Proc R Soc B* 272, 2155-2163.

318. Castro G., Myers J. P., Ricklefs R. E. 1992 Ecology and energetics of sanderlings migrating to 4 latitudes. *Ecology* 73, 833-844.

319. Nagy K. A. 2005 Field metabolic rate and body size. *J Exp Biol* 208, 1621-1625.

320. Martin L. B., Gilliam J., Han P., Lee K., Wikelski M. 2005 Corticosterone suppresses cutaneous immune function in temperate but not tropical House Sparrows, *Passer domesticus*. *Gen Comp Endocrinol* 140, 126-135.

321. Hutchinson G. E. 1957 Concluding remarks: Cold Spring Harbor symposium. *Quantitataive Biol* 22, 415-427.

322. Angelier F., Weimerskirch H., Dano S., Chastel O. 2007 Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behav Ecol Sociobiol* 61, 611-621.

323. Newsome S. D., del Rio C. M., Bearhop S., Phillips D. L. 2007 A niche for isotopic ecology. *Front Ecol Environ* 5, 429-436.

324. Lambrechts M. M., Adriaensen F., Ardia D. R., Artemyev A. V., Atienzar F., Banbura J., Barba E., Bouvier J. C., Camprodon J., Cooper C. B.*, et al* 2010 The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. *Acta Ornithologica* 45, 1-26.

325. Klein A., Nagy T., Csorgo T., Matics R. 2007 Exterior nest-boxes may negatively affect barn owl *Tyto alba* survival: an ecological trap. *Bird Conserv Int* 17, 273-281.